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Emotional Changes and Lesions of "Temporal Lobe" Structures

By Carmine D. Clemente*

RELATIVELY LITTLE ATTENTION was directed at first to a theory proposed by Papez in 1937[1] outlining a possible anatomic and functional approach to an understanding of the "emotions." In the past 10 years, however, it has become increasingly apparent that relay systems such as the hippocampus, the hypothalamus, the anterior thalamic nuclei, the septum, the cingulate cortex and their connections are functionally intimately interrelated when one attempts to apply an organic neural substrate to problems dealing with behavior and emotional expression. Even with this more modern realization, many so-called scientific discussions of problems involving the "mind," the "personality" and the "psyche" are divorced from brain structure and function. Basic scientists such as Judson Herrick[2] pointed out long ago that rhinencephalic structures in the brain probably not only serve an olfactory function but also were important regions which influenced behavioral patterns and exerted central visceral control.

A brief glimpse into the clinical literature also will indicate that lesions in limbic areas and temporal lobe structures give rise to "altered inner feelings," "emotional disturbances" and "marked changes in behavior." Temporal lobe seizures, either of the psychomotor or subjective (psychic) types, have been described as associated with an onset of a marked emotional feeling. Excessive fear is one of the most frequently encountered emotional signs described by patients with temporal lobe pathology and seizures. Jackson and Stewart[3] described a fear that "came by itself" in a patient thought to have a temporal lobe tumor. Since then, many authors have reported on patients having temporal lobe tumors or other temporal pathologic involvement in which feelings of fear and undefinable terror ushered in the psychomotor seizure.

Another emotion described in the clinical literature in relation to temporal lobe disease is uncontrollable anger and fierce outbursts of rage. Cases reported both in adults and in children implicate limbic area lesions in this emotional expression. It is interesting that van der Horst's[4] patient showed furious and violent emotional discharges and that the tumor involved the septum, fornix, hippocampus, hypothalamus and thalamus, in striking anatomic similarity to the proposed emotion mechanism of Papez[1] expressed nearly 20 years before. A feeling of euphoria and well-being is only infrequently encountered with temporal lobe involvement.

With respect to expressions of sexual feelings and temporal lobe lesions in humans, several fascinating cases have been reported in the recent clinical literature. In 1955, Terzian and Dalla Ore[5] described the case of a 19-year-old man with a history of psychomotor and *grand mal* seizures. When the patient was placed under observation, he displayed periods of aggressive, violent behavior. On the basis of EEG localization, he was operated on, and the entire anterior portion of the left temporal lobe was removed. Postoperatively, his psychomotor attacks reappeared and no improvement was observed in his behavior. Nineteen days later, his right temporal lobe was removed in order to modify his violent episodes. Soon after the second operation, the patient demonstrated an insatiable appetite, eating as much as four normal people. He then developed sexual aberrations in the form of exhibitionism and homosexual advances toward his doctor. He displayed no interest in females. Although there were serious defects in his memory, he no longer manifested rage. His generalized epileptic attacks reappeared after two months, but he no longer had psychomotor seizures. Pen-

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field[6] described a patient with a lesion in the temporal lobe who exhibited sexual ideas as a component of his dreamy states.

The clinical literature cited above is only a minute representation of the available clinical data relevant to the problems under discussion here. With the availability of the clinical literature, and with the theory of emotion proposed by Papez, it is not surprising that basic neurophysiologists have rediscovered the importance of temporal lobe areas and subjacent nuclear structures in an analysis of a physiologic basis for emotional expression. In animal experimentation, reports of aberrant behavior following temporal lobe lesions exist in the literature since the work of Brown and Schäffer in 1888.[7] These investigators observed a state of relative docility, seeming "idiocy," hyperphagia and exaggerated oral behavior following bilateral temporal lobotomy in adult monkeys. Klüver and Bucy,[8-10] Gastaut,[11] and Schreiner and Kling[12-14] described aberrations in sexual behavior in monkeys and cats after temporal lobe lesions that also destroyed the underlying amygdala-pyriform areas. The striking homosexual, heterosexual and indiscriminate behavior observed by these authors in monkeys and cats with bilateral temporal lobectomies were not observed following unilateral temporal lobe lesions or in animals with "control" lesions in other cerebral lobes.

Schreiner and Kling, in addition to observing manifestations similar to those of Klüver and Bucy in cats and monkeys following bilateral removal of the piriform-amygdala-temporal lobe complex, also describe tandem copulation and copulation with other species. These investigators believed that endocrine secretions from the gonads maintained the hypersexuality, since the abnormal behavior was abolished by castration. Injection of testosterone after gonadectomy restored the hypersexual behavior to precastration levels.[13]

One of the difficulties in the interpretations of such experiments is in the relative paucity and weakness of the "control" observations with respect to the "experimental" observations. With this realization in mind, J. D. Green, J. de Groot and I intensively studied the sexual behavior in a series of over 40 normal cats for periods of up to 6 months as a background of comparison against a series of 82 animals that sustained lesions in various areas of the limbic system. Whereas previous investigators removed the entire temporal lobe bilaterally along with the amygdaloid nuclei and pyriform cortex, we have been able to destroy selectively smaller portions of the amygdala and adjacent regions by stereotaxically placing electrolytic lesions. In this way we tried to analyze the "Klüver-Bucy" syndrome into its different components.

These findings have been published in detail elsewhere,[15,16] but several of the more interesting findings will be discussed here in the light of the observations of others. First of all, it was most surprising to learn that intact male cats with no lesions in the central nervous system would exhibit many of the bizarre "hypersexual" behavior patterns following temporal lobe lesions described by other authors. After a five- to seven-day period, when the male cat was presented with an estrus female daily in an observation cage, he became conditioned and adapted to "territory." After adaptation, he would carry out sexual relations with any receptive female immediately upon presentation. The adapted male cat would also carry out heterosexual, homosexual and tandem (three animals or more) copulation in much the same manner as the animals with lesions described by Schreiner and Kling.[12]

"Territory" can be any room or part of the laboratory, so long as the surroundings presented to the male cat during adaptation are exactly the same each time. When a male cat that has adapted to one "territory" is placed in completely unfamiliar surroundings, he no longer will carry out these strange behavioral patterns unless he is allowed to adapt to the new area. It seems as though "territory" adaptation is related to a feeling of familiarity of the area and feelings of confidence while in the area.

The meaningfulness of the "territory" concept was most important in our analysis of hypersexuality in animals with limbic lobe lesions. Male hypersexual behavior was induced by bilateral electrolytic lesions limited to the pyriform cortex underlying (i.e., ventral to) the amygdaloid nuclei. Such animals would perform all the acts noted in animals with intact brains, in addition to attempts at copulation with other species and inanimate objects. More important, however, was the finding that in animals with lesions, "territory" had no meaning, and upon being placed in unfamiliar surroundings, these animals would display all the behavioral characteristics seen in "territory." Indiscrimination prevailed with respect to sex of partner, place, species of animal and length of copulatory period.

These experiments indicate that the so-called "state of hypersexualism" in animals described

by some authors cannot be accepted without stringent control studies in which the limits of "normal" sexual behavior have been ascertained. They also show that both entire temporal lobes do not have to be destroyed in order to produce the behavioral aberrations; smaller bilateral lesions limited to more specific regions (pyriform cortex) can result in hypersexuality. Since the lesions in most of the cases involving temporal lobe lesions described in the clinical literature are unilateral, it is not surprising that hypersexual manifestations have rarely been observed to complicate cases of psychomotor epilepsy.

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On Editors, from the Point of View of an Author

By Milton Mendlowitz*

I HAVE READ many editorials and treatises on the plight of the poor editor and what he has to contend with from authors whose papers he has to rewrite, from disgruntled readers, from printers who hang him on deadlines, and, as an author, I sympathize. But in the midst of this compassion, the question occurs to me of why he became an editor in the first place? He made the bed, after all, and he probably made it because he likes the down of prominence, ego support and contact with the ideas of his peers, as well as for the more often expressed reason of eagerness to advance his field by continuous critical and often agonizing reappraisal.

Mutual sympathy in the interest of politeness between editor and author can, therefore, I think, be dispensed with and the situation viewed realistically. The fact is that a relationship of antagonism more than of love often prevails. When a paper is submitted to a journal for publication the emotions aroused on both sides of the fence are more apt to be something like this:

Author: "I think I will send this paper to Editor X, but if he submits it to Reviewer Y on whose toes we tread, God knows what will happen to it. Besides, he thinks anything written is automatically bad unless it comes from an Authority, and I will probably have to fight this one all the way down the line."

Editor: "My God, is Z sending in a paper again? It is so complicated I can't even begin to read it, much less understand it; and it's probably way out in left field anyway. What can one expect from someone coming from Hospital A? How can I let him down easy?"

Of course, if the paper is one requested by the editor, the author can write almost anything he chooses and the poor editor is stuck with it. This is why one finds some of the most uncritical and unchallenged effluvia in symposia and reviews so requested. A good way in which to have an unacceptable idea published by the editor willy-nilly, therefore, is to arrange to big panel symposium, all of which is contracted to be published. The wild thoughts that are expressed in discussions and papers in this kind of program would be hacked to pieces if submitted individually by the authors. This, then, is one way that the author has to say what he pleases, provided he is invited to the symposium to start with, and wishes to be invited to others.

Another method by which an editor is frequently nudged to accept a paper against his better judgment is if it is submitted by his own superior or by someone sponsored by this personage or, again, by someone powerful enough for his anger to matter. The editor may here, unless he is made of very stern stuff, be influenced to yield a little.

Most often, however, the problem is the other way around. What is the easiest way to let the author down but keep him thinking that you (the editor) are noble and fair minded, even if he knows that you really think the work is not good enough for your journal?

There are several techniques for doing this sort of thing, all failures, as a rule. The editor may say that the paper is too technical for his readers and suggest that it be submitted to a more basic (sic) journal. Or, he may say that the paper is excellent but is too long and would have to be broken up into four or five papers which he might not be able to publish, so perhaps it should be sent to a journal that accepts long papers. Or, that this is a short paper which deserves rapid publication and he has a two-year backlog. And one could go on.

A more prevalent device, and one gaining favor, is the reviewer system, which is a subject unto itself. The editor takes the modest position that after all he doesn't know everything. He therefore refers papers in certain areas to members of his editorial board or to friends who are allegedly authorities in this field. They then proceed to clobber the paper with relevant or irrelevant criticisms which are sent to the author who now becomes so frustrated and discouraged that he figures, "the hell with it." This lets the editor off the hook because, first of all, he doesn't have to do much work, and, second, he takes

*The Mount Sinai Hospital, New York, N. Y.

the position that he has to abide by the opinions of his experts. What is more, the author can't hate anyone, since the editor is impartial and the reviewers anonymous.

On the face of it, this seems a foolproof protective armor for the editor, unless the author has the courage of his convictions and, thus baited, fights back. He then sends in a blistering commentary on the reviewers' comments, and this may go back and forth several times.

If the editor is experienced, he usually remains impartial in this branigan, but sometimes he makes the mistake of getting into it. He may thus reveal some weaknesses, in biostatistics, for example, if he is a clinician, or in clinical medicine, if he is a basic scientist. This does not matter very much if only one benighted author observes it; but it can get around.

Now what motivates the reviewers, who are really the editors in this situation? They must show the editor that they are highly critical. They like or don't like the author personally. The paper agrees or does not agree with what they have written on the subject. The author fails to quote the reviewer or his friends. The reviewer is writing a paper on the same subject and what right has the author to publish his paper first? The author is a full-time or a part-time worker or an ivory-tower scientist or an ignorant clinician. These reasons all come into play, in addition to the laudable motive of giving every paper a fair shake.

The cloak of anonymity protecting the reviewers is, moreover, not impenetrable. Very often the author can detect from the names on the editorial board and the style of the comments who the reviewer is, especially if there are only a few *cognoscenti* in a given area of research. This is even more frustrating, because he cannot be absolutely sure unless the editor lets something slip during the course of the correspondence.

There are several types of reviewers' comments that are particularly irritating to authors. First, the comment which obviously reveals that the reviewer has neither read the paper carefully nor taken the trouble to understand it. Second, the reviewer who criticizes with an opinion instead of offering facts to the contrary. In other words, it sounds impressive, but he just doesn't believe it. Or, again, the reviewer who says he doesn't see why the paper was written at all (since he didn't think of it first!). And perhaps the most irritating thing of all to have happen is to comply with revisions suggested by reviewers and then have the paper refused anyway—it now being obvious that the original suggestions were merely made so that the author would go away.

The general effect of this is to delay the publication of papers for months and often years, especially if you add on the editor's vacation time or his trip to India or Europe as government envoy, or his just forgetting all about a manuscript on the shelf in the hope that the author will forget that he submitted it. Despite the fact that by this time the author's paranoid trends have become highly exaggerated, it is not uncommon for a paper so delayed or even roundly trounced by one journal to be accepted without a murmur by another and even require immediate correction of proof to make next month's issue.

Having been not only author but also, at times, reviewer, and at least editorial writer if not editor, I see the problems on both sides of the issue, or at least so I hope. Let it be said then that many editors are good men trying to do a good job, and perhaps the end result of all this is to improve the quality of the papers published, although this is often not readily apparent. The system can only be fought by authors if they let no reviewer's comments, which they consider unfair, go unanswered, regardless of whether the paper is acceptable to the journal in the end or not. In this way the editor becomes educated and can eventually choose a board of reviewers only on the basis of criteria which it is hoped he has for himself.

Fortunately, also, there are so many journals available, so many editors, of which are men of good will, that despite viscissitudes and storms, no good paper need be completely shipwrecked if the author has the hardiness to steer his course and, of course, if he lives long enough.

NOTICES

Call for Abstracts
National Meeting, May 3, 1959

The 16th Annual Meeting of the American Federation for Clinical Research will be held in the Casino Theater on the Steel Pier, Atlantic City, New Jersey on Sunday, May 3, 1959. There will be a general session from 9 A.M. to 5 P.M. In addition, subspecialty meetings will be jointly sponsored by the Federation and the American Society for Clinical Investigation. The exact topics for these meetings will be determined by the president from the number of abstracts received in the various fields of interest.

All papers submitted to the Federation will be considered for the general session on Sunday. Those not included in the general session program will be submitted to the chairmen of the subspecialty meetings as determined by the president. All abstracts should be sent to: Albert I. Mendeloff, M.D., Sinai Hospital of Baltimore, Baltimore 5, Maryland.

Abstracts must be postmarked not later than February 19, 1959. Air mail should be used whenever delivery by regular mail cannot be accomplished within 36 hours.

The paper may not be simultaneously submitted to the American Society for Clinical Investigation. Any paper which is submitted to both organizations will automatically be disqualified from both programs. Members should carefully heed this rule, since it represents a change in policy.

(Abstracts may not be withdrawn after February 26).

Authors are reminded to follow the rules applying to abstracts which are listed below.

Rules Applying to Abstracts

1. The title should be brief, with capitalization only of initial letters, and no capitals in prepositions, conjunctions, or articles. It should clearly indicate the nature of the investigation. It should be followed by the names and institutional affiliations (if no institution is involved, only the city should be given) of the authors. Authors' names should be underlined. Degrees, titles, institutional appointments, and membership status are omitted. Support of the work by a research grant will not ordinarily be mentioned.

The title and identifying material should be double spaced at the top of the first page of the abstract. A separate title page should *not* be used. This model should be exactly followed:

The Effect of Nitrate Ingestion on the Ammonia Content of Hepatic Venous Blood

By John W. Throckmorton and Angus McBeth. Sixth Medical Service, City Hospital, West Pines, Georgia, and Department of Medicine, Busby Medical School.

2. The body of the abstract must not be longer than *250 words*, organized in the following manner:

(a) A brief statement of the purpose of the study (preferably one sentence).

(b) A statement of the methods used.

(c) A summary of the results obtained.

(d) A statement of the conclusions reached. It is not satisfactory to say, "The results will be discussed."

3. The text should be intelligible without reference to tables or graphs, which *cannot be published* in this journal.

4. The use of standard abbreviations (RBC, for example) is desirable. This journal uses Kg., Gm., mg., μg., cc., L. (for liter), mEq., M. (for meter), and the per cent sign (%). A special or unusual abbreviation should be followed by a parenthetical explanation the first time it is used. Numerals, rather than words, should in general be used to indicate numbers, except in beginning sentences.

5. Each time the proprietary name of a drug is used in title or text, the first letter should be capitalized. Nonproprietary names should be written without capitals.

6. Abstracts, *including titles*, should be double spaced. The original and *five* copies must be submitted. The original should be on ordinary white bond paper; contrary to previous instructions, the copies should be on thin paper.

7. Each abstract must be accompanied by a covering letter giving the name, address, age and membership status of each of the authors and stating which author will present the paper. No one who has passed his forty-first birthday may present the paper.

8. One of the authors must be a member of the Federation, or the paper must be introduced by a member.

9. For the National Meeting, a member of the Federation may either introduce one paper *or* submit one paper. He may, however, also be a co-author of a paper submitted by another member.

Bertha Goldblatt Teplitz Award

The Ann Langer Cancer Research Foundation announces the Bertha Goldblatt Teplitz Award of $500 annually for meritorious investigation in the field of cancer research, either clinical or laboratory. Those eligible are physicians and other scientists, clinical or laboratory, under the age of 45. The winner of the 1959 award will be announced on May 1, 1959. Nominations should be submitted to: Teplitz Award Committee, 612 North Michigan Avenue, Chicago 11, Illinois by February 1, 1959, and should be accompanied by a one-page statement and biography.

Application for Membership

1. Young research workers are encouraged to apply for membership. It is unnecessary to await a member's invitation to join the AMERICAN FEDERATION FOR CLINICAL RESEARCH.

2. There is one requirement for regular membership: publication of a meritorious investigation in clinical medicine or allied sciences. This should not be a case report or an abstract.

3. An applicant must ask a member of the Federation who knows him to sign his application.

4. Interested individuals should write to: AMERICAN FEDERATION FOR CLINICAL RESEARCH, 250 West 57th Street, New York 19, N.Y.

New Research Tools

The information reported here is obtained from manufacturers. All notices and inquiries should be addressed to **New Research Tools Editor, Samuel N. Turiel & Associates, Inc., 750 N. Michigan Ave., Chicago 11, Ill.** Include name(s) of the manufacturer(s).

• **Enzyme listing** with descriptive technical data. Nutritional Biochemicals Corp.

• **Scintillation detector** for counting fast or slow neutrons, and beta radiation, one-inch diameter and weighing less than 5 ounces. Resolution time in the microsecond region permits use in high radiation fields. Accessories include a neutron probe, and three needle probes, suitable for surgical applications, for measuring radiation in areas difficult of access. Nuclear-Chicago Corp.

• **Microscope slide** for preparation of cytologic smears, divided into three separate spaces. Owens-Illinois.

• **Scaler** that can be set for both present time and present count simultaneously. Tracerlab, Inc.

• **Avidin,** 5-hydroxytryptophane, S-carbamyl-L-cysteine, glyoxylic acid, 6-phosphogluconate (barium), 2 aminoethylisothiourea dihydrobromide, and 6 azathymine available. Nutritional Biochemicals Corp.

• **New miniature pH electrode assembly,** for use with samples of one drop or larger. Self-shielding of measuring electrode eliminates compartmental shielding and need for sample transfer. pH range is 1 to 11.5. Temperature range for sample 15 to 40°C. Leeds and Northrup Co.

• **Fractionator** which collects fractions directly in rectangular test tube racks. Racks can be removed with test tubes in place in their boustrophedon order. Unit provides constant volume output per sample, and any volume between 1 and 30 cc. may be collected. Each unit equipped with 3 test tube racks, each containing 5 rows of 15 tubes. Gilson Medical Electronics.

PROGRAM, EASTERN SECTION

American Federation for Clinical Research

Friday and Saturday, December 12 and 13, 1958
Jimmy Fund Building Auditorium, Children's Medical Center
Boston, Massachusetts

Dr. William B. Schwartz, Presiding

Presentations will be limited to ten minutes, with ten minutes for discussion.

FRIDAY, DECEMBER 12

9:00 A. M.

1. **The Effect of Strophanthidine on Electrolyte Metabolism and PAH Accumulation in Rabbit Kidney Slices.**
   Maurice Burg and Jack Orloff, Bethesda, Md. *page 42*
2. **Glomerular Perfusion during Acute Renal Insufficiency from Mercury Poisoning in the Rat.**
   *Ethan A. H. Sims,** Herbert I. Goldberg,* Joseph R. Kelley* and Burton A. Sisco,** Burlington, Vt. *page 43*
3. **The Effect of Antidiuretic Hormone on the Permeability of the Toad Bladder.**
   Roy H. Maffly, Richard M. Hays, Ezra Lamdin and Alexander Leaf,* Boston. *page 44*
4. **Mechanisms of Hyperlipemia in Experimental Nephrosis.**
   *Norman Kalant and Judith Saffran,** Montreal, Quebec. *page 44*
5. **The Effect of a Low-Potassium Diet on the Renal Response to Respiratory Acidosis.**
   Howard Levitin, David Beck and Franklin H. Epstein, New Haven, Conn. *page 42*

INTERMISSION

6. **Pyrimidine Biosynthesis in Leukemia.**
   *Lloyd H. Smith, Jr. and Faith Baker,** Boston. *page 14*
7. **Neonatal Neutropenia due to Maternal Isoimmunization.**
   Parviz Lalezari, Murray Nussbaum,* Sidney Gelman* and Theodore H. Spaet,* New York. *page 13*
8. **The Effect of 6-Mercaptopurine on Immune Responses.**
   Robert Schwartz, Anna Eisner, and William Dameshek,*** Boston. *page 39*
9. **The Effect of Purine Nucleosides on the Reduction of Methemoglobin in Human Erythrocytes.**
   Ernst R. Jaffé, New York. *page 12*
10. **The Mechanism of Action of Hemolysis-inducing Drugs on Glucose-6-Phosphate Dehydrogenase.**
    Ester Kalaw and Jane F. Desforges,* Boston. *page 11*

12:30 P. M.

LUNCHEON (for Members)
Gymnasium, Vanderbilt Hall
Harvard Medical School
Courtesy of Parke, Davis & Co.

AFTERNOON SESSION

2:00 P. M.

Dr. Kurt J. Isselbacher, Presiding

11. **Antibodies against Nucleoprotein Extracts in Patients and Animals.**
    *Howard C. Goodman and Robert Bowser,** Bethesda, Md. *page 40*
12. **Localization of the Effect of Intrinsic Factor in the Rat Small Intestine in Vitro.**
    Victor Herbert, Zaida Castro and Louis R. Wasserman,** New York.* *page 32*
13. **An Experimental Malabsorption Syndrome Induced by Neomycin.**
    Eugene D. Jacobson, Robert B. Chodos*

*By Invitation
**Senior Member

and William W. Faloon, Syracuse, N. Y. *page 33*

14. **Effect of Parathyroidectomy on the Early Distribution of Radiocalcium in Rats.**
*Lawrence G. Raisz and David F. Hammack,** Syracuse, N. Y. *page 24*

15. **Isolation of Cortisol from a Pheochromocytoma.**
*Patrick J. Mulrow** and George L. Cohn,* West Haven, Conn. *page 29*

INTERMISSION

16. **Effect of Glucagon on Homeostatic Balance between Hepatic and Peripheral Carbohydrate Metabolism.**
*Dorothy H. Henneman and William Shoemaker,** Jersey City, N. J. *page 25*

17. **Differential Resin Binding of Insulin in Serum.**
*Marvin L. Mitchell,** William O. Whitehead** and Mary E. O'Rourke,** Boston. (Introduced by *Bruce C. Ferguson.*) *page 26*

18. **Is There an Abnormal Thyroid Hormone-Plasma Protein Complex in Graves' Disease?**
John B. Richards, J. Thomas Dowling and Sidney H. Ingbar, Boston. *page 23*

19. **The Effect of Antithyroid Substances on the Intrathyroidal Metabolism of Iodine.**
*D. Ward Slingerland,** Eiichi Yamazaki,** Roma K. Josephs,** Ann Trakas,** Dorothy E. Graham** and Philip Mulvey,** Boston. (Introduced by *Belton A. Burrows.*) *page 24*

COCKTAIL PARTY: Courtesy of Merck, Sharp and Dohme
and
DINNER: Courtesy of Pfizer Laboratories
(for Members)
LONGWOOD TOWERS
Corner of Longwood Avenue and Chapel Street, Brookline

EVENING SESSION
8:00 P. M.

PANEL DISCUSSION

What is the Future of the "Clinical Investigator"?
Participants: Philip K. Bondy, M.D., Associate Professor of Medicine, Yale University School of Medicine; Editor-in-Chief, The Journal of Clinical Investigation.

Charles A. Janeway, M.D., Professor of Pediatrics, Harvard Medical School.

Edward H. Kass, M.D., Associate Professor of Bacteriology and Immunology, Harvard Medical School.

Irving M. London, M.D., Professor of Medicine, Albert Einstein College of Medicine.

John M. Russell, Executive Director, John and Mary R. Markle Foundation.

Moderator: William B. Schwartz, M.D.

SATURDAY, DECEMBER 13

9:00 A. M.

BUSINESS MEETING

9:15 A. M.

Dr. Daniel S. Lukas, Presiding

20. **The Effect of Potassium on the Inotropic Action of Cardiac Glycosides.**
*Edward Leonard** and Stephen Hajdu,** Bethesda, Md. (Introduced by Thomas J. Kennedy, Jr.) *page 19*

21. **Pressor Effects of Digitalis Glycosides.**
*John Ross, Jr.,** John A. Waldhausen,** James A. McFarland** and Eugene Braunwald,* Bethesda, Md. *page 20*

22. **A Principle for Quantifying Regional Blood Flows.**
*Gilbert E. Levinson,** Walter H. Abelmann and Leon Cudkowicz,** Boston. *page 22*

23. **Percutaneous Splenic Pulp Manometry as an Aid in the Diagnosis of Acute Upper Gastrointestinal Bleeding.**
*William F. Panke,** Augusto H. Moreno,** Louis M. Rousselot** and William J. Grace,*** New York. *page 38*

24. **Measurement of Ventricular-Atrial Regurgitation in Dogs by Indicator Dilution Technics.**
*Lucien Arditi,** Charles W. Pearce, Arnold L. Winston and Daniel S. Lukas,* New York. *page 16*

25. **An Objective Technic as an Aid in the Diagnosis of Acute Pulmonary Embolism.**
*Eugene D. Robin, David M. Travis, Desmond G. Julian** and Charles H. Crump,** Boston. *page 47*

26. **Pulmonary Pressure-Volume Relationship in Thoracic Deformity (Kyphoscoliosis).**
*Er Yi Ting and Harold A. Lyons,*** Brooklyn, N. Y. *page 47*

Expenses of this meeting were partially defrayed by grants from: *Lakeside Laboratories, Inc., Smith, Kline and French Laboratories, The Upjohn Company.*

TO READERS AND CONTRIBUTORS

An Experiment in Medical Journalism

The policy of Clinical Research is not only to give rapid publication to research abstracts, but also to provide a vehicle for kinds of articles which do not ordinarily find places in other publications.

These articles should be informed but brief statements of *views*, and they may cover a wide variety of professional matters. Their subject matter will range from purely scientific questions to discussions of research in general and of the environment in which research is carried on. Some of them will be solicited, but we hope that interested authors will submit such material of their own accord. The views expressed may well be somewhat partisan, and we expect that they will evoke counter-statements by workers who are not in agreement with them. A conscientious effort will be made to publish as many of these statements as space allows in early succeeding issues. Such contributions are of course subject to the customary editorial discretion.

Summary reviews of the usual type and original research communications (apart from abstracts) will not ordinarily be acceptable for the Journal, since numerous publication opportunities for such contributions already exist.

The aim of the policy is to provide a meeting place for medical minds, such that the membership of the American Federation for Clinical Research, and other interested persons, may benefit from the enormous amount of careful thought, unsupported by specific laboratory data, that is now being given to important professional issues by competent and conscientious workers. We would like our content to be often controversial without being contentious, and to point occasionally to worthwhile objectives without crusading.

The Editor and his associates solicit the advice and good will of readers of Clinical Research. The development of this experiment in medical journalism must depend on the willingness of competent persons to express themselves in print, and also on the willingness of readers of conviction to write in opposition to or in support of communications in the Journal. Such expression of opinion may take the form of "Letters to the Editor," and it is our hope that there will be an active Correspondence Section. Suggestions for changes in plan or for new activities will be welcomed.—*David T. Graham*

Advance Reports Submitted to the Annual Meeting of the EASTERN SECTION of the American Federation for Clinical Research

Jimmy Fund Building Auditorium, Boston, Massachusetts
Friday and Saturday, December 12 and 13, 1958

BLOOD

Measurement of the Production and Life Span of Erythrocytes in Myeloid Metaplasia

By *David G. Nathan and Nathaniel I. Berlin.* Metabolism Service, National Cancer Institute, Bethesda, Maryland.

Anemia may be a major cause of disability in myeloid metaplasia. The study of sites of erythropoiesis and red cell destruction may be of importance in therapy. This paper presents the results of 4 such studies which included measurements of plasma and red cell iron turnover with Fe^{59}; serial in vivo measurements of Fe^{59} in bone marrow, liver and spleen; the apparent red cell survival with Cr^{51}; the rates of accumulation of Cr^{51} in liver and spleen in vivo; and the red cell life span with glycine-2-C^{14}.

The patients had active splenic, hepatic or renal erythropoiesis. One had deficient erythropoiesis in all organs. The red cell life span was short in all patients due to random destruction of variable intensity with definite evidence of splenic sequestration in 2 patients. Evidence was found in one case for active splenic red cell production with failure to discharge a significant fraction of the cells into the peripheral blood.

The Mechanism of Action of Hemolysis-inducing Drugs on Glucose-6-Phosphate Dehydrogenase

By *Ester Kalaw and J. F. Desforges.* Tufts Hematology Laboratory, Boston City Hospital, Boston.

Patients suffering from certain drug-induced hemolytic anemias are deficient in glucose-6-phosphate dehydrogenase. The present investigation was conducted to determine whether these drugs inhibit this enzyme and to study the kinetics involved.

Enzyme inhibitors may be classified as competitive or noncompetitive. The type of inhibition is determined by mathematical observations of reaction velocity with varying substrate concentration.

Incubation of glucose-6-phosphate dehydrogenase (G-6-P D) with sulfanilamide, primaquine and nitrofurantoin resulted in competitive inhibition. In contrast, menadione sodium bisulfite, parachloromercuribenzoic acid and B-naphthol exhibited noncompetitive inhibition. There was no evidence of competition with triphosphopyridine nucleotide (TPN).

The effect of acetylphenylhydrazine is also to inhibit G-6-P D, but its insolubility made it impossible to determine the type of inhibition.

6-Phospho-gluconic dehydrogenase (6-P-G D) inhibition was also investigated, since this is the other TPN-dependent dehydrogenase in the glycolytic cycle and is sequentially related to the

G-6-P D reaction in the hexosemonophosphate shunt. With this enzyme, sulfanilamide, menadione sodium bisulfite and nitrofurantoin were found to be competitive inhibitors, and primaquine noncompetitive.

It is apparent that the drugs which are known to cause hemolytic anemia in susceptible individuals and in individuals with deficient G-6-P D may act as inhibitors to this enzyme system. The mechanism of reaction of these different drugs is not the same. Sulfanilamide and similar compounds may compete with the substrate for the active site in the coenzyme molecule which is the para-position of the pyridine ring, while nitrofurantoin, which has an entirely different chemical structure, may act through interference with electron transfer. Noncompetitive inhibitors such as B-naphthol may inactivate the prosthetic group of the apoenzyme by inactivating SH groups.

The kinetics of enzyme inhibition and the Michaelis constants both demonstrate that the mechanism reaction of these two TPN-dependent dehydrogenases is not the same.

In summary, in vitro observations have demonstrated that certain drugs which cause hemolytic anemia or hemolysis in vitro act as direct inhibitors of G-6-P D and also of 6-P-G D. The inhibition is either competitive or noncompetitive.

The Effect of Purine Nucleosides on the Reduction of Methemoglobin in Human Erythrocytes

By *Ernst R. Jaffé*. Department of Medicine, Albert Einstein College of Medicine, and Bronx Municipal Hospital Center, New York City.

The property of normal human erythrocytes to reduce methemoglobin (Methgb.) to hemoglobin (Hgb.) upon incubation with various substrates has been utilized to study the influence of nucleosides and related compounds on Methgb. conversion. Whole blood was incubated (40 min., 37 C.) with sufficient sodium nitrite to oxidize 60–80% of the Hgb. to Methgb. The erythrocytes were washed 3 times with isotonic sodium chloride solution, and 25% suspensions in this medium were prepared. To 2 ml. of isotonic sodium chloride-phosphate buffer solution, pH 7.3, containing the compounds under study were added 4 ml. of erythrocyte suspension, and these suspensions, whose pH was 7.3–7.4, were incubated with shaking at 37 C. Aliquots of these suspensions were removed at intervals for estimation of Methgb. and lactic acid.

Significant reduction of Methgb. to Hgb. occurred upon incubating normal erythrocytes with the following compounds at 8–10 μm./ml. of packed erythrocytes: purine ribosides (adenosine, guanosine, inosine, 2,6-diaminopurine riboside, xanthosine), purine deoxyribosides (deoxyadenosine, deoxyguanosine, deoxyinosine), sugars (glucose, fructose, galactose, ribose, deoxyribose, L (+)-sodium lactate) and ascorbic acid. Ineffective compounds included nucleotides, pyrimidine nucleosides, adenine glucoside, adenosine-1-N-oxide, purine riboside, adenine, several phosphorylated intermediates of carbohydrate metabolism and miscellaneous compounds. Similar experiments performed with erythrocytes from a patient with congenital methemoglobinemia demonstrated that of the compounds effective in normal erythrocytes only ascorbic acid resulted in Methgb. conversion, although amounts of lactic acid comparable to those observed with normal erythrocytes were produced.

The metabolism of various sugars and the metabolism of the pentose moiety of purine nucleosides by human erythrocytes may result in the regeneration of reduced pyridine nucleotides that are involved in Methgb. conversion. The lack of effect of these compounds in erythrocytes of congenital methemoglobinemia is consistent with the concept that the defect lies in electron transport between reduced pyridine nucleotides and Methgb.

Methemoglobin Reduction and Erythrocyte Glucose-6-Phosphate Dehydrogenase Activity

By *J. Ross and J. Desforges*. Tufts Hematology Laboratory, Boston City Hospital, Boston.

Incubation of erythrocytes containing methemoglobin with glucose and methylene blue normally results in an efficient reduction of methemoglobin. This reduction is believed to be dependent upon reduced triphosphopyridine nucleotide (TPNH). Since one of the two sources of TPNH in the human erythrocyte is the oxidation of glucose-6-phosphate in the presence of glucose-6-phosphate dehydrogenase (G-6-P D), it was decided to attempt to correlate the activity of that enzyme in the erythrocyte with the ability of the erythrocyte to reduce methemoglobin in the presence of glucose and methylene blue. Blood from normal adults, from glutathione in unstable subjects and from umbilical cords was

studied. Erythrocytes were exposed to sodium nitrite to form methemoglobin, washed free of excess nitrite and incubated for 180 minutes in the presence of glucose and methylene blue. Methemoglobin concentrations were determined at zero hours and at 3 hours. G-6-P D activity of hemolysates from these subjects was measured by observing the rate of appearance of TPNH in the presence of glucose-6-phosphate and TPN.

In normal bloods, the amount of methemoglobin reduced was proportional to the initial methemoglobin level. This was therefore expressed as per cent of the zero hour methemoglobin reduced. Methemoglobin reduction by the erythrocytes of subjects with normal G-6-P D activity was normal in all cases.

The G-6-P D levels of cord bloods was elevated or high normal in the majority of samples. A small number were decreased below normal limits and represented a genetic enzyme defect. In the cord blood samples, the methemoglobin reduced was roughly proportional to the glucose-6-phosphate dehydrogenase activity. In 3 of 4 cord bloods with deficiency of G-6-P D, the reduction of methemoglobin was also deficient. Methemoglobin reduction by a 4th cord blood with decreased G-6-P D activity was normal. In one case, methemoglobin reduction was deficient in the presence of brisk TPNH generation.

Reduction of methemoglobin by erythrocytes of 5 of 7 Negro subjects with known glutathione instability and deficiency of G-6-P D was deficient.

The process of methemoglobin reduction by the TPN-TPNH pathway involves multiple steps. The results of these studies demonstrate that the available TPNH may be a limiting factor in this process. The lack of correlation between TPNH production and a methemoglobin reduction in certain cases demonstrates that other factors may play the determining role in controlling the rate of the final reaction.

Hemoglobin-binding Capacity of Plasma in Sickle Cell Disease

By *Wallace N. Jensen and Willoughby Lathem.* Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh.

The capacity of plasma proteins to bind hemoglobin has been found to be decreased or absent in a small percentage of normal individuals, in some patients with hepatic disease and in certain hemolytic disorders. The mechanism, specificity and significance of the reduced binding capacity in hemolytic states have not been established. These considerations prompted the investigation of hemoglobin binding in a group of hemolytic, hemoglobinopathic states not previously studied.

Using a quantitative in vitro method, the binding of hemoglobin was studied at hemoglobin concentrations of 100 and 200 mg. % in patients with sickle cell disease (SS), sickle cell trait (SA) and hemoglobin C disease (CC). Plasma hemoglobin was partitioned by paper electrophoresis (pH 7.0), and the individual fractions were quantified by photometric analysis following staining with benzidine-hydrogen peroxide.

The binding of both normal and abnormal hemoglobin was quantitatively normal in sickle cell trait but was greatly reduced or absent in sickle cell anemia and hemoglobin C disease. Since the binding of hemoglobin S and hemoglobin C by normal plasma was quantitatively normal, these alterations are attributable to a disturbance in the binding capacity of plasma proteins, the concentration of which is apparently reduced.

The mechanism of this reduction was not established but may be attributed to alterations in the rate of productions of these proteins and/or in the rate of their removal from plasma.

Under these in vitro conditions, a small quantity of methemalbumin was found in normal plasma. Greater amounts of methemalbumin were present in those plasma samples where hemoglobin-binding capacity was reduced. This may be attributable to the higher concentrations of free hemoglobin present in the plasma when hemoglobin-binding proteins are absent.

Neonatal Neutropenia due to Maternal Isoimmunization

By *Parviz Lalezari, Murray Nussbaum, Sidney Gelman and Theodore H. Spaet.* Department of Hematology, Laboratory Division, Montefiore Hospital, New York, and Department of Medicine, Seton Hall College of Medicine, Jersey City, New Jersey.

A family is reported with multiple cases of neonatal neutropenia. The first child died at the age of 9 days of bronchopneumonia with severe neutropenia. The second child had an uneventful neonatal period, and white cell counts are unavailable. The third child developed severe cellulitis of the scalp at 12 days of age and was found to be severely neutropenic. Following antibiotic

therapy and, subsequently, use of steroids, clinical and hematologic recovery occurred. The youngest child developed omphalitis at 8 days of age and was likewise noted to be neutropenic. She is gradually recovering following treatment of the infection with antibiotics. There is no family history of similar disease, and the mother denies previous transfusions or miscarriages.

The maternal serum showed potent leukoagglutinins active against cells of all living children and the husband. No abnormal antibodies against erythrocytes were detected. The serum of the youngest child also had leukoagglutinating activity but of different specificity, in that it agglutinated the leukocytes only of the father and one sibling; the patient's own cells and those of the other sibling were not agglutinated. At the time of study, neutropenia was still present.

It is believed that this represents a condition of neutrophiles analogous to hemolytic disease of the newborn.

Pyrimidine Biosynthesis in Leukemia

By *Lloyd H. Smith, Jr. and Faith Baker.* Department of Medicine, Massachusetts General Hospital, and Harvard Medical School.

There has been a continuing interest in nucleic acid metabolism in neoplasia because of the evidence that deoxyribonucleic acids (DNA) and ribonucleic acids (RNA) are concerned with genetic transmission and protein synthesis, respectively. In addition, structural analogues of various purines and pyrimidines have proved to be partially effective experimentally and clinically as antineoplastic agents.

Methods have been developed for the quantitative assay in circulating leukocytes of 3 enzymes—aspartate carbamyltransferase, dihydroorotase and dihydroorotic dehydrogenase—leading to the formation of the first pyrimidine, orotic acid. These enzymatic activities have shown reproducible levels when expressed per unit number of normal cells. In leukemia, all of these enzymatic activities are increased per cell, particularly dihydroorotic dehydrogenase which may be 4–10 fold increased. Enzymatic levels compare favorably with those found in rat liver per mg. N. The enzyme 5-carboxymethylhydantoinase, present in some bacteria in the pyrimidine synthetic pathway, was absent from both normal and leukemic human leukocytes.

Several members of a new class of antimetabolites, structural analogues of carbamylaspartate, have been synthesized and found to inhibit competitively the conversion of carbamylaspartate to orotic acid in vitro. The use of enzymes from human leukocytes to test the potential effectiveness of these antimetabolites has been illustrated.

Culture of Bone Marrow on Dextrose Agar

By *Retha Odom and Edward H. Reisner.* Department of Medicine, St. Luke's Hospital, New York.

Bone marrow, cultured by a method (Clin. Res. Proc. 5:145, 1957) providing conditions of nutrient factors and gas interchange less favorable than those classically employed, will maintain its identity and produce recognizable blood cells for periods up to several months. We believe that in these cultures the cells differentiate because the environment is not optimal for division.

To study the effect of sub-optimal culture environment on maturation vs. division, human marrow explants were grown on dextrose agar slants (Sabouraud's) with no additional nutrient. After 24 hours, migratory cells invaded the surrounding medium, giving the explant a "halo" appearance which persists throughout the life of the explant. Collecting enough of the migratory cells for qualitative study has proven difficult because the agar dissolves during fixing and the cells are lost. H and E sections of the explant have an appearance typical of bone marrow with all cell lines represented for from 4 to 8 weeks. After this there is an increasing number of undifferentiated cells, and at 90 days the marrow contains neutrophile and eosinophile myelocytes, some segmented granulocytes, reticulum cells and lymphocytes or plasma cells. No fibroblasts are seen. Clumps of hemosiderin are present, implying that the iron from disintegrating erythrocytes is deposited in the explant but not reutilized for hemoglobin synthesis. Preparing the agar with malignant exudate, mixture 199, or a 60–40 mixture of both did not produce any significant difference in growth pattern or types of cells observed. Preliminary studies of explants on gelatin without any additives are encouraging, and observations are similar to results on agar. These observations suggest that for the slow rate of division characteristic of marrow in vivo, the nutrient supplied by the explant itself is sufficient for long periods of time.

Further Studies on the Activation of Purified Prothrombin

By *Liberto Pechet, Garson H. Tishkoff and Benjamin Alexander.* Medical Research Laboratories, Yamins Laboratory, Beth Israel Hospital, and Department of Medicine, Harvard Medical School, Boston.

The conversion of prothrombin to thrombin by certain anions requires convertin complex and/or some other nonprothrombin entity associated with convertin in its purification. A convertin-rich serum fraction exposed to citrate will activate purified proconvertin-poor prothrombin, which alone cannot be activated. This suggests that anion activation, as in the biologic pathway, is mediated through convertin rather than by direct action on prothrombin. The present study was aimed at further elucidation of convertin in prothrombin activation, and further resolution of the convertin-rich fraction.

A purified convertin fraction was shown to be partially stable to 5% trichloracetic acid; 25–30% of its activity was recoverable following precipitation with TCA. In striking contrast, its ability to activate prothrombin after citrate exposure was completely destroyed. This suggested that prothrombin activation by citrate-triggered convertin-rich fractions was mediated through a nonconvertin substance, labile to TCA.

Further separation of the components of a prothrombin preparation contaminated with proconvertin was attained by starch block electrophoresis. The electrophoretic fraction containing peak prothrombin activity was devoid of convertin activity as well as thrombin. As expected, this prothrombin failed to yield thrombin with citrate in contrast to the original material. Moreover, a faster migrating convertin-rich fraction was obtained which was free of prothrombin. This fraction did not generate thrombin with citrate.

Recombination of the prothrombin and convertin fraction and subsequent exposure to citrate failed to yield thrombin. Thrombin was obtained only in the presence of prothrombin, convertin and a third component which migrated even faster, although close to the convertin fraction. This component was free of thrombin. Although its identity is still obscure, the data indicate further complexity in the anion pathway of prothrombin activation.

Studies continue on the nature of this third moiety and its role in the biologic prothrombin conversion pathway.

The Effect of Heparin on Thromboplastin Generation

By *Jacob N. Shanberge.* Departments of Pathology, Harvard Medical School, Boston, and V. A. Hospital, West Roxbury, Massachusetts.

It has been shown by several investigators that heparin, by inhibiting the formation of thromboplastin, interferes with the conversion of prothrombin to thrombin. No definite explanation for this inhibition had been offered. The following, therefore, are the results of some of our studies of the effect of heparin on thromboplastin activity as produced in the thromboplastin generation test of Biggs and Douglas, as well as its effect on the various components of the generation mixture.

Heparin markedly interferes with the formation of blood thromboplastic activity as well as "formed" thromboplastin. This inhibition of thromboplastin generation can be counteracted by increasing the concentration of normal serum in the generation mixture, but not by increasing the concentration of the adsorbed plasma or platelet thromboplastic factor. The inhibition of thromboplastin generation by heparin can also be counteracted by protamine sulfate.

Serum from an individual with a congenital or acquired deficiency of PTC (Factor IX) either does not counteract the influence of heparin or reacts in inverse proportion to the severity of the deficiency. From this, one may infer that heparin interferes with the conversion of prothrombin to thrombin in shed blood by interference with whatever is responsible for PTC activity.

CARDIOVASCULAR SYSTEM

Previously Unrecognized Atrial Potentials of High Frequency and Low Voltage

By *Cesar A. Caceres and George A. Kelser, Jr.* Department of Medicine, George Washington University Hospital, Washington, D.C.

Left and right atrial intracavitary and conventional surface leads were used to study electrocardiographic activity during the PR interval. Electronic filters were employed for analysis of wave frequency and harmonic content from 0.1 to 2000 cycles per second. Amplifiers permitting standardization sensitivities to 500 mm./mv. were used to obtain oscilloscopic tracings recorded at a paper speed of 75 mm./sec.

Frequency analysis of the electrical potential recorded during P wave inscription demonstrated the presence of high frequency content that is excluded by conventional electrocardiographic amplifiers.

A spike of electrical activity of high frequency is noted approximately 0.03 to 0.04 second after onset of the limb lead P wave. The high frequency activity is recordable from the right atrium and occurs temporally with the rise of pressure in that chamber. A similar spike of high frequency activity may be seen when the electrode is in the left atrium, but it occurs near the end of the limb lead P wave and occurs temporally with the onset of left atrial pressure. Although the intracavitary P wave pattern and its intrinsic deflection vary with location of electrode within the atrium, the time of occurrence of high frequency activity remains constant.

The presence of these previously unrecognized spikes of high frequency activity after onset of atrial depolarization suggests that extremely rapid electrical changes are associated with activation of certain portions of myocardial tissue during specific instants of the cardiac cycle. The time of occurrence of the spikes in the cardiac cycle correlates with sino-atrial and atrio-ventricular nodal events. The occurrence of the spikes at the onset of atrial pressure rise suggests a relationship to the initiation of contractile events.

Measurement of Ventricular-Atrial Regurgitation in Dogs by Indicator Dilution Technics

By *Lucien Arditi, Charles W. Pearce, Arnold L. Winston and Daniel S. Lukas.* Department of Medicine and Cardio-Pulmonary Laboratory, New York Hospital-Cornell Medical Center.

The reliability of indicator dilution methods for measuring valvular regurgitation has been verified in circulation models but remains to be defined in vivo. Accordingly, these methods were assessed in dogs with left ventricular-atrial regurgitation produced surgically by a shunt from the apex of the left ventricle to the left atrial appendage.

The rate of regurgitation could be varied at will and was monitored constantly by a ½ in. Potter electroturbinometer. Rates up to 3.3 L./min. were attained.

A technic utilizing injection of T-1824 at a constant rate into the left ventricle and measurement of dye concentration in left atrial and aortic blood at equilibrium was assayed in 10 dogs. In 39 trials the values obtained by this technic failed to correlate flow meter values and in general grossly underestimated the rate of regurgitation. The poor results were attributed to incomplete mixing in the ventricle and atrium. They cast doubt on the validity of measurements by the left ventricular injection—left atrial sample technic in man.

In an additional 7 dogs, dilution curves were recorded from the aortic arch via cuvette oximeter following rapid "single shot" injection of T-1824 or indocyanine green into the left atrium. In 50 instances, rates of regurgitation calculated from the ratio of reciprocal of down slope of regurgitant curve to reciprocal of slope of control curve (recorded with shunt closed) correlated very closely with the flow meter rates, and in the majority of instances agreed within 0.3 L./min. In the calculations, it was found necessary to use control curves derived for each animal. Other characteristics of the curves, i.e., variance, minimum concentration/recirculation concentration, were less useful in measuring regurgitation.

These observations strengthen the contention that the terminal slope of an indicator dilution curve is a reliable index of the rate of regurgitation and stress the necessity of using a control curve in the calculation.

Application of a New Variance Regression Equation for the Quantification of Mitral Regurgitation

By *Richard A. Carleton and Gilbert E. Levinson.* Thorndike Memorial Laboratory and Second and Fourth (Harvard) Medical Services, Bos-

ton City Hospital, and Department of Medicine, Harvard Medical School.

The variance method of Korner and Shillingford (Clin. Sc. *15*:417, 1956) for the quantification of valvular incompetence has been reappraised. Treating indicator dilution curves as frequency distributions, and using variance to express dispersion, they demonstrated in a circulatory model that dispersion is determined by volume and flow. A regression equation relating these factors was constructed. Regurgitation was associated with an increased variance, which was related to the diminished probability of forward flow, permitting the quantification of regurgitation in the model. Similarly, they derived an equation from subjects without valvular incompetence and utilized this to compute the regurgitant volume in patients.

In this laboratory, the physiologic assessment of mitral valve disease is by left heart catheterization. Applying the equation of Korner and Shillingford to data from left atrial dye (T-1824) injections, we obtain physiologically improbable volumes of regurgitation for most patients. There are, moreover, theoretical objections to the application of this equation to data obtained by different technics and from different injection and collection sites than were employed in the original work.

For these reasons, we have derived a new regression equation for left atrial indicator dilution curves. The equation is:

$$\log \text{predicted variance} = 2.6303 - 2.0637 \log CO + 1.8799 \log V$$

where CO is forward flow in L./min. and V is "central volume" in liters. With this equation we have calculated the regurgitant volume in 49 patients (56 curves) with isolated mitral valve disease.

The results are compared with an independent clinical and surgical estimate of the severity of mitral incompetence. The values from the new equation give a significant positive correlation, a better separation of the clinical groups and a reduction of scatter within each group.

This means of estimation of valvular incompetence appears to have great clinical utility despite the lack of an absolute standard of comparison.

The Diagnosis of Ostium Primum Defects

By *Herbert Mark and Dennison Young*. Medical Division, Montefiore Hospital, New York City.

The characteristic clinical findings associated with A.S.D. (atrial septal defect) enable one to make a diagnosis on clinical grounds alone with a high degree of accuracy. An ostium primum type of lesion with persistence of the A.-V. (atrioventricular) canal may produce a similar clinical picture. Surgical technics for the open repair of these congenital cardiac defects are available. However, closure of an ostium primum defect with an associated defect of an A.-V. valve is a considerably more difficult procedure than repair of an uncomplicated ostium secundum, and at times may not be feasible. The present study was undertaken to ascertain whether pre-operative differentiation between these lesions is possible. Twenty-two cases of surgically or pathologically proven A.S.D.'s (secundum type) and 6 ostium primum lesions with and without persistent A.-V. canal have been included. Clinical, electrocardiographic, roentgen and physiologic findings were available for comparison.

Auscultatory findings were similar in both groups and of no differential value. Congestive heart failure and cyanosis were noted in the more seriously ill patients of both groups, and significant pulmonary hypertension was usually demonstrated in the same patients. Pulmonary artery pressures tended to be higher in the smaller group of patients wtih ostium primum lesions.

The roentgen picture was of value when enlargement of the left ventricle could be unequivocally demonstrated.

Electrocardiographic signs were of the greatest differential value. Left axis deviation with right ventricular hypertrophy or right bundle branch block occurred in 5 of the 6 patients with ostium primum lesions, whereas ostium secundum lesions were associated with the usual pattern of incomplete right bundle branch block and/or right ventricular hypertrophy. Ostium primum and secundum defects and a cleft mitral cusp were demonstrated in the 6th patient whose electrocardiogram showed a right axis deviation and right ventricular hypertrophy pattern only. One patient with a left axis deviation on electrocardiogram had an ostium primum without a mitral valve defect.

Prolongation of the P-R interval was noted, although this is a less reliable sign.

It is concluded that the electrocardiographic pattern of left axis deviation and right ventricular hypertrophy or right bundle branch block in the presence of clinical atrial septal defect is considered diagnostic of ostium primum lesions with or without further endocardial cushion defects.

Pharmacologic Aspects of Ventricular Fibrillation under Hypothermia

By *E. T. Angelakos, E. Hastings and A. H. Hegnauer.* Department of Physiology, Boston University School of Medicine, Boston.

Over the last 3 years, the effect of several pharmacologic agents on the hypothermic heart has been studied in over 1000 dogs. These experiments have been primarily directed toward elucidating the mechanism of ventricular fibrillation (VF) under hypothermia. Nevertheless, the accumulated experience has brought forth certain points which may find some applicability in man under clinical conditions.

A large body of experimental data will be shown to substantiate the following conclusions: (1) Spontaneous hypothermic VF (without any manipulation of the heart) is prevented to a large extent by quinidine and certain antihistaminics, especially antistine, chlorothen and methapyrilene. Procaine amide is of no value in this case and in fact may precipitate VF at a relatively high temperature. (2) The VF which occurs during surgical manipulation of the heart can be prevented to a large extent with maintenance of normal blood pH combined with quinidine or antistine. Treated animals are apt to develop an atonic heart after a period of venous inflow occlusion and terminate in heart failure if other measures are not taken. This is most marked with antistine. (3) Electrical defibrillation is successful under hypothermia provided the heart is not atonic. Defibrillated animals (treated or controls) may subsequently develop atonic hearts. (4) The development of an atonic heart in all hypothermic animals can be prevented to a large extent by previous digitalization, but it is best treated with intracardiac epinephrine injections and massage. With such treatment it seems to be entirely reversible. (5) Digitalization does not appear to alter the susceptibility of the hypothermic heart to VF. In fact, hypothermia reduces digitalis toxicity.

The Effects on Myocardial Oxygen Availability of Hemorrhagic Shock and its Reversal by Various Agents, Including L-Norepinephrine

By *Francis S. Caliva, Rudolph Napodano, Robert Zurek, Tony Pombo and Richard Lyons.* Department of Medicine, Upstate Medical Center, State University of New York, Syracuse, New York.

The purposes of this study were 2-fold: (1) to study the effects of induced hypotension and shock on myocardial oxygen availability, and (2) to determine if the adverse effects, if any, could be reversed by agents such as l-norepinephrine which act to raise the arterial pressure and also, possibly, to dilate coronary vessels.

Oxygen availability, as measured by intramyocardial platinum electrodes, femoral artery pressures and limb electrocardiograms were continuously and simultaneously recorded in 19 dogs who were bled to shock levels. Blood pressure levels were subsequently restored by the administration of either l-norepinephrine, whole blood, plasma or dextran.

There was found to be a very close correlation between oxygen availability and mean arterial pressure. Between 30 and 90 mm. Hg, this relationship was almost linear. Above and below these levels, there was suggestive flattening of the curves. At approximately 20 mm. Hg, oxygen availability was at the same levels as those recorded when the animal's heart had stopped beating. Restoration of blood pressure by any of several methods uniformly resulted in return of oxygen levels towards normal. Changes in heart rate were variable and did not correlate well with oxygen changes. The EKG's were unremarkable except for findings consistent with "ischemia."

It is concluded that myocardial oxygen availability and probably also "effective" coronary blood flow decrease with falling blood pressures. Restoration of arterial pressure reverses these changes, and l-norepinephrine seems very adequate in this respect.

Pyridoxine Deficiency in Congestive Heart Failure

By *Howard A. Levy, Michael G. Wohl and Carl Alper.* Hahnemann Medical College, Philadelphia.

The investigation was carried out to determine whether pyridoxine (B_6) deficiency occurs in patient with congestive heart failure due to organic heart disease, since it has already been demonstrated that thiamine deficiency occurs in these patients after a 24-hour urine control collection. Tryptophan load tests, using a single 10 Gm. dose of d,l tryptophan, were carried out before and after a 50 mg. therapeutic dose of B_6 given parenterally and the amount of xanthurenic acid in a 24-hour urine collection estimated by a modification of the method of Poseu

et al. There were 20 controls, including hospitalized patients in the same age groups as the 20 patients with obvious clinical evidence of congestive heart failure. The latter included arteriosclerotic, hypertensive and rheumatic heart disease. All patients were placed on a 1.0 Gm. sodium chloride hospital diet known to contain an average of 2 mg. pyridoxine daily. In addition, they were given 10 mg. thiamine HCl acid, 5 mg. riboflavin daily, since coenzymes containing these vitamins are known to be involved in the metabolism of tryptophan. Signs of overt malnutrition or undernutrition were noted in only 2 patients in the control group. In the control group, the 24-hour urinary xanthurenic acid excretion rose from 5.5 mg. (1.0–12.1) control to 7.3 mg. (2.7–12.9) after tryptophan loading and then fell to 4.0 mg. (1.8–7.5) after pyridoxine administration and tryptophan loading. The heart failure cases showed 4.7 mg. (1.3–11.6) control, 40.0 mg. (11.3–109.5) after tryptophan loading, and 8.8 mg. (2.9–19.0) after pyridoxine and tryptophan loading. Since it is generally accepted that the presence of excessive amounts of xanthurenic acid in the urine is indicative of pyridoxine deficiency, these results strongly indicate that a deficiency of the vitamin occurs in congestive heart failure. The rapid return to a normal output of xanthurenic acid following a large dose of pyridoxine tends to confirm these conclusions.

Experimental Congestive Heart Failure: Bioenergetic Studies

By *Menard M. Gertler*. New York University-Bellevue Medical Center, Institute of Physical Medicine and Rehabilitation, New York City.

Bioenergetic differences have been studied between normal guinea pig hearts and guinea pig hearts in which congestive heart failure was produced experimentally.

Congestive heart failure has been produced in the guinea pig by surgical means. This experimental congestive heart failure has been shown to involve sequentially the left ventricle and atrium, the lungs, the right ventricle and atrium, liver and other organs.

Mitochondrial systems derived from the heart and liver in both normal and congestive heart failure animals were studied as follows: (a) the efficiency of phosphorylation in relation to oxidation (P/O ratio); (b) oxidation per hour, per mg. of nitrogen ($Q^N o_2$) value; (c) use of inhibitors, such as malonate.

It was demonstrated that the P/O ratios with alpha ketoglutarate or glutamate as substrates were within the normal range for the congestive heart failure animals in both the liver and heart mitochondrial systems. The $Q^N o_2$ values for liver and heart in the congestive heart failure group were lower than in the normal group.

The addition of malonate to the heart and liver mitochondrial systems from the congestive heart failure animals with alpha ketoglutarate as the substrate had striking results. The $Q^N o_2$ value compared to normal animals is depressed 60% and 90% in the liver and heart, respectively. The P/O ratios could not be calculated. The malonate inhibitory effect on the $Q^N o_2$ value could be reversed in both the liver and heart mitochondrial systems by the addition of certain combinations of co-factors. Although the co-enzymes restored the $Q^N o_2$ values, phosphorylation remained depressed, indicating either that irreversible damage had been done to the energy-yielding process by malonate in congestive heart failure mitochondrial or that additional factors may have to be added for its restoration.

The Effect of Potassium on the Inotropic Action of Cardiac Glycosides

By *Edward Leonard and Stephen Hajdu*. Laboratory of Kidney and Electrolyte Metabolism, National Heart Institute, Bethesda, Maryland.

Despite the fact that the positive inotropic action of cardiac glycosides is associated with loss of potassium from heart muscle, and despite reports that increasing serum potassium may reverse manifestations of glycoside toxicity, it has not been established whether increasing extracellular potassium inhibits glycoside-induced cellular potassium loss or whether it stops the disturbances in rhythm without altering the inotropic action. This study was done to find whether the inotropic action of glycosides on isolated cardiac tissue was inhibited by increasing extracellular potassium concentration. In determining inotropic effects, the frequency of muscle stimulation must be controlled, since contractile force rises as the rate of stimulation is increased over a certain range. A positive inotropic effect is manifested by a decrease in the stimulus frequency required to maintain a given twitch tension. Using this sensitive criterion, it would be expected on the basis of previous studies that interventions causing a net loss of intracellular potassium would be associated with a positive inotropic effect and

vice versa. For frog heart in the absence of glycoside, extracellular potassium concentration can be decreased from the normal 2.5 mM/L. to 1.25 or increased to 5.0 mM/L. without significantly altering the contractile force-stimulus frequency relationships, which suggests that potassium can be varied over this range without causing a significant net change in intracellular potassium. Only as extracellular potassium is decreased below 1.25 mM/L. is a positive inotropic effect observed. The action of strophanthidin on force-frequency relationships for heart muscle of frog, guinea pig and rabbit was not significantly altered by increasing potassium concentration from 2.5 to 5.0 mM/L. for frog and from 4.7 to 7.5 mM/L. for mammalian tissues. It is concluded that the positive inotropic action of strophanthidin is not inhibited by increasing extracellular potassium concentration over the range studied.

Pressor Effects of Digitalis Glycosides

By *John Ross, Jr., John A. Waldhausen, James A. McFarland and Eugene Braunwald.* National Heart Institute, Bethesda, Maryland.

There is increasing evidence for an extracardiac action of the digitalis glycosides. A direct pressor effect of digitalis has been suspected but has not been clearly separated experimentally from its powerful inotropic effect. Total body perfusion with complete exclusion of the heart and lungs permits precise evaluation of extracardiac vascular reactions.

Cardiopulmonary bypass was established utilizing a rotating disc oxygenator and a non-occlusive roller pump. Total systemic flow was maintained constant and was continuously monitored with an electromagnetic flow meter. In some experiments, femoral artery flow was measured with a recording rotameter, and in others, hepatic venous outflow was measured directly.

In all 10 dogs, an elevation of arterial pressure occurred following the administration of 0.06 mg./Kg. of ouabain. Since systemic perfusion was constant, this represented an elevation of peripheral resistance. The average maximum increase in total peripheral resistance was 60%, with a range from 24% to 118% above control values. Elevation of the vascular resistance in the limb occurred in 3 of the 4 animals in which femoral artery flow was monitored. In all experiments, the maximum pressor effect was observed within 30 minutes following ouabain administration.

Paper Electrophoresis and Liver Function Studies in Chronic Congestive Failure

By *Reuben Schucher and Joseph Wener.* Biochemistry Laboratory and Department of Medicine, Jewish Hospital, Montreal.

Paper electrophoresis and liver function tests were performed on a group of 27 patients with long-standing chronic congestive failure (duration of failure 2–10 years). These patients were well nourished and were treated with moderate salt restriction (5–6 Gm./day) and liberal diuretic therapy.

Over 90% of the patients had at least one or more abnormalities in hepatic tests; the highest percentage of abnormal findings was with the B.S.P. excretion (70%). The serum bilirubin level was normal in all cases. The serum total protein was normal for all patients (mean 7.7 ± 0.65 [S.D.] Gm.%). Electrophoretic fractionation of the sera gave the following results in Gm.%: albumin 4.6 ± 0.79 (S.D.), α_1 0.33 ± 0.05, α_2 0.58 ± 0.3, β 0.83 ± 0.14, γ 1.38 ± 0.46. The results were the same as those obtained with a control group of 25 healthy members of the hospital staff.

The percentage of abnormalities in the flocculation tests were similar to those reported by others. Despite the presence of abnormal liver tests in over 90% of the patients, not a single abnormality was found in the total serum proteins or in the electrophoretic fractions, contrary to reports of hypoalbuminemia and increases in one or more of the various globulin fractions in congestive failure.

From these results, one may consider that liberal protein intake may well be the major factor in maintaining normal serum protein levels, despite the high percentage of impaired liver function tests, in patients with long-standing congestive heart failure.

Increased Incidence of Coronary Heart Disease in Prematurely Castrated Women

By *Norio Higano, William D. Cohen and Roger W. Robinson.* The Memorial Hospital, Worcester, Massachusetts.

Coronary heart disease is rare in premenopausal women. The increased incidence postmenopausally has been ascribed to decreased ovarian estrogen production. A disproportionate number of middle-aged women with coronary heart disease under our care had been prematurely castrated. These observations prompted an

investigation to determine whether or not early castration plays a significant role in coronary atherogenesis. During the past 20 years, about 400 patients have undergone bilateral oophorectomy prior to age 45 at this hospital. Two hundred and four of these women responded to a request for clinical and laboratory evaluation. Of these, 130 without previous prolonged estrogen replacement therapy comprised the study group. A control group consisted of 135 women who had undergone hysterectomy without castration during the same period. The mean ages at operation and at recall for examination of the castrates and controls were nearly identical. Obesity was present equally in both groups. An apparent increased prevalence of hypertension in the castrates (65; 50%) as compared with the controls (58; 43%) was not statistically significant. The incidence of vascular retinopathy, cardiac enlargement, peripheral vascular disease, migraine, diabetes, thyroid diseases, gall bladder disease and miscarriages was practically identical in the 2 groups. Seventeen of the 130 castrates (13%) were found to have coronary heart disease, in contrast to 6 of the 139 controls (4%). This difference was highly significant, suggesting that estrogen deprivation after bilateral oophorectomy is associated with a subsequent increased incidence of coronary heart disease. Thus, early castration of women should be avoided unless specifically indicated.

Oligemia as Basic to Chlorothiazide Action in Hypertension

By *C. A. Macleod, Harriet P. Dustan and R. E. Schneckloth.* Research Division, Cleveland Clinic Foundation, and Frank E. Bunts Educational Institute.

In our early experience with chlorothiazide, the enhanced responsiveness to ganglioplegic drugs seemed dependent upon a decrease in plasma volume, but a specific antipressor effect was not eliminated. Results of subsequent experiments, summarized below, using chlorothiazide alone, emphasize the importance of oligemia and support a new concept of the mechanism of action of chlorothiazide in hypertension. In these studies, plasma volume (PV) was estimated from the volume of distribution of I^{131} albumin. Cardiac index (CI) was determined by dye dilution, using indigo-carmine. Supine and standing blood pressure (BP) was measured 4 times daily.

In 12 patients given chlorothiazide, 30 mg./Kg./day for 3 days, PV decreased daily; decreases of standing BP were oserved by the 2nd day of the study. At the end of 3 days, percentile changes for the group were: PV −12%, mean supine BP −4%, mean standing BP −10%, body weight −2.4%, urinary sodium excretion +98%.

In 9 patients, 4–6 day chlorothiazide treatment decreased PV 14%, CI 24%, mean blood pressure (MBP) 6% and increased total peripheral resistance (TPR) 23%. Tetraethylammonium chloride (TEAC), 5 mg./Kg., was given intravenously to 6 of these patients. Without chlorothiazide, TEAC depressed CI 20%, decreased MBP transiently 8% and elevated TPR 25%. During chlorothiazide, TEAC decreased CI 20%, decreased MBP 24% and, instead of increasing, now decreased TPR 8%.

The diuresis and saluresis of chlorothiazide cause oligemia. Direct measurements in animals show that oligemia increases vasomotor tone. The present data support the view that chlorothiazide-induced oligemia in hypertensive patients augments vasomotor outflow and thus enhances the antihypertensive effects of drugs affecting the autonomic nervous system; however, in some susceptible patients, it may, itself, be antihypertensive.

Experimental Embolization Using Clots Labelled with Cr^{51}

By *Philip E. Duffy and Frank W. Furth.* Department of Medicine, State University of New York, Upstate Medical Center, Syracuse, New York.

During the course of studies on experimental cerebral embolization, a method was devised to label clots with Cr^{51}. This permitted quantitative observation of the clot following its introduction into the circulation of dogs.

One ml. of freshly drawn dog blood mixed with 100 μc. of $Na_2Cr^{51}O_4$ was drawn into polyethylene tubing and allowed to coagulate. Eighteen hours later the clot was rinsed with saline to remove loosely adherent red cells. A segment of known dimensions of this clot was placed in a 2.0 cm. length of plastic tubing. A femoral artery of a dog anesthetized with Nembutal was exposed and clamped at 2 levels. The tubing containing the tagged clot was completely inserted into the isolated section of artery and fixed by ties. The radioactivity of the clot was measured using a scintillation detector. The clamps were then removed and the progress of the embolus in the artery was followed by means

of the scintillation detector. At points of greatest activity, repeated determinations of radioactivity were made over a period of 2 hours. During this same period, peripheral blood samples for radioactivity assay were obtained from the external jugular vein. Periodic determinations of radioactivity over the area of the spleen were also obtained.

Twelve dogs were studied with this technic. Following release of the embolus, radioactivity was found concentrated in one or more isolated peripheral areas in the limb. At the insertion site, radioactivity decreased immediately to background levels. Approximately 50% of the original radioactivity in the clot disappeared from the peripheral sites within 20 to 40 minutes. This was accompanied by a reciprocal increase in the radioactivity of the jugular blood. A slower rate of decrease in local radioactivity over the peripheral site occurred during the following 2 hours. Within 2½ hours, less than 20% of the original radioactivity in the clot remained at the peripheral sites. Within 30 to 90 minutes the radioactivity in the jugular blood reached a maximum. A subsequent slow decrease in peripheral blood radioactivity was accompanied by an increase in radioactivity over the spleen.

During the period of observation no radioactivity was found in the plasma. From these data it is evident that the Cr^{51}-tagged red cells became separated from the embolus. The release of greater than 80% of the cells from the embolus suggests that actual dissolution of the clot occurred. Although fibrinolysis was not directly measured, this technic represents a useful method for studying the dynamics of clot dissolution and experimental embolization.

A Principle for Quantifying Regional Blood Flows

By *Gilbert E. Levinson, Walter H. Abelmann and Leon Cudkowicz.* Thorndike Memorial Laboratory and Second and Fourth (Harvard) Medical Services, Boston City Hospital, and Department of Medicine, Harvard Medical School.

If, following rapid venous or central injection of an indicator, a vessel V receives blood flow from 2 or more sources among which is the complete outflow from region R, and if indicator from R arrives prior to indicator from other sources, an early curve, representing regional flow, will be inscribed, following mixing, at or distal to V. It can be shown that the regional flow

$$\dot{Q}_R = \frac{\dot{Q}_R + \dot{Q}_V}{\frac{A}{A_V}}, \text{ where } \dot{Q}_V = \text{flow, other than}$$

$\dot{Q}_R$, traversing V, A = the area under the conventional arterial dye dilution curve, and A_V the area under the early regional curve. Since, whenever V is a cardiac chamber, $\dot{Q}_R + \dot{Q}_V$ is the output of either the left or right side of the heart, this principle can be applied to at least 3 systems: (1) left-to-right shunts; (2) coronary flow; and (3) broncho-pulmonary anastomotic flow.

Flow through septal defects should produce early and discrete contributions to the dye curves obtained at right ventricle or pulmonary artery after injection into distal pulmonary artery or left atrium. The short, low volume pathway from coronary artery to coronary sinus should produce an early contribution to the curve inscribed from pulmonary artery after injection into the left atrium. Flow through anastomoses between bronchial vessels and the pulmonary arterial or venous circulation should produce an early discrete contribution to the dye curve obtained from left atrium after injection into left ventricle or that obtained from left ventricle after injection into aorta.

In vivo validation of this principle has been obtained through studies of the latter system. Flow through broncho-pulmonary anastomoses has been estimated both by this principle and as the difference between simultaneously measured left and right ventricular outputs, with satisfactory agreement between the two methods.

ENDOCRINES AND METABOLISM

Is There an Abnormal Thyroid Hormone-Plasma Protein Complex in Graves' Disease?

By *John B. Richards, J. Thomas Dowling and Sidney H. Ingbar.* Thorndike Memorial Laboratory, Second and Fourth (Harvard) Medical Services, Boston City Hospital; Department of Medicine, Harvard Medical School, Boston; and Howard Hughes Medical Institute.

It has been suggested that in Graves' disease there exists an abnormality in the thyroid hormone-plasma protein complex. This hypothesis is largely based upon increased uptake of I^{131}-labelled thyroxine and triiodothyronine by cellular systems incubated in thyrotoxic sera.

In addition to inter-alpha globulin (TBG) and albumin, which can be shown to bind thyroxine during paper electrophoresis of serum in veronal buffer, an additional, highly avid thyroxine-binding protein (thyroxine-binding pre-albumin, TBPA) has recently been demonstrated in this laboratory during electrophoresis in tris-maleate buffer. Thyroxine-binding by TBPA is usually decreased in thyrotoxic patients. However, comparable decreases also occur in a variety of non-thyroidal illnesses. When binding by TBPA is diminished, thyroxine binding by the relatively weak carrier, albumin, is increased.

These findings prompted a re-examination of the "erythrocyte uptake" of thyroxine from the sera of normal, thyrotoxic and other sick patients. Studies were performed at endogenous levels of PBI and following enrichment of sera from non-thyrotoxic patients to levels of PBI comparable to those of thyrotoxic subjects. A close correlation was found between decreased binding by TBPA and the magnitude of the "erythrocyte uptake." Thus, in normal serum, uptakes at endogenous PBI levels were low and were little increased by enrichment of the PBI. Uptakes at endogenous PBI levels were moderately increased in sera from both thyrotoxic and non-thyrotoxic sick patients. Uptakes were even further increased in the latter group when sera were enriched to thyrotoxic concentrations of PBI.

These data do not support the concept that there exists in patients with Graves' disease a specific abnormality in the thyroid hormone-plasma protein complex. Rather, they suggest that the increased "erythrocyte uptake" of Graves' disease is conditioned by the concentration of PBI but dependent upon a reduction in thyroxine-binding by TBPA. The latter abnormality is shared by many patients with non-thyroidal illness.

Effect of Tri-Iodothyropropionic Acid on Circulating Lipids of Euthyroid Adults

By *Louis E. Schaefer and David Adlersberg.* Department of Medicine, Mt. Sinai Hospital, New York City.

Tri-iodothyropropionic acid (TP), an analog of tri-iodothyronine, was given orally in doses of 1 to 8 mg. per day (average 2.5 mg.) for 1 to 10 months (average 4.5 months) to 17 euthyroid adults, age 27 to 73 years. Five patients had previous myocardial infarctions (2 hypercholesteremic), 5 asymptomatic idiopathic hypercholesteremia, and 7 were "normal." The subjects were examined monthly; their lipids and BMR were studied at regular intervals. Diet was kept constant during both control and experimental periods. An average of 3 pretreatment serum lipid determinations were obtained. Ten patients were normocholesteremic according to age- and sex-corrected standard (Adlersberg et al. J.A.M.A. *162*:619, 1956). The average duration of treatment was 4.2 months. Mean serum cholesterol decreased from 269 to 234 mg.% (−13%), range 0 to −23%. Serum phospholipid changes were less constant, but mean cholesterol/phospholipid ratio decreased from 0.93 to 0.81 (−12%).

Seven patients were hypercholesteremic by the same standards.. Average duration of treatment was 4.7 months. Mean serum cholesterol decreased from 353 mg.% to 289 (−18%), range 0 to −44%. Serum phospholipid change was again less constant, but mean cholesterol/phospholipid ratio decreased from 1.06 to 0.87 (−18%). In one patient, extensive lid xanthelasma disappeared after 6 months of treatment.

Except in one patient who developed thyrotoxicity after 4 months (but became euthyroid 2 months after cessation of treatment), there was no evidence of significant calorigenic effect. None of the 5 with coronary disease developed angina. No "escape" from the effect with time was observed, although this phenomenon had been noted with tri-iodothyronine analogues.

TP seems to be effective in lowering serum cholesterol and cholesterol/phospholipid ratio in a majority of euthyroid patients, although more

extensive evaluation is necessary. The effect appears to be greater in hypercholesteremic subjects. It is not known whether the effects on serum lipids obtained are sufficient to alter the course of atherogenesis.

The Effect of Antithyroid Substances on the Intrathyroidal Metabolism of Iodine

By *D. Ward Slingerland, Eiichi Yamazaki, Roma K. Josephs, Ann Trakas, Dorothy E. Graham and Philip Mulvey.* Boston V. A. Hospital, and Tufts University, School of Medicine.

Antithyroid compounds block the binding of iodine to tyrosyl radicals in thyroglobulin. A possible effect of antithyroid compounds at another step in the intrathyroidal metabolism of iodine was studied. A day after injection of radioiodine (5–100 cc.) into rats fed control and test diets (1–11 days) until 24 hours before injection, the thyroids were removed, hydrolysed with pancreatin and chromatographed.

The ratio of monoiodotyrosine to diiodotyrosine (MIT/DIT) was 0.48 in the control, 2.3 in propylthiouracil-(PTU) treated animals. The ratio of triiodothyronine to thyroxine (T_3/T_4) was 0.2 in the control, 1.6 in the PTU-treated animals. The content of radioiodine was 38% of the control value in PTU-fed rats; in individual animals, however, the content was often equal to the control, and the change in MIT/DIT still occurred. The addition of thyroid hormone (TH) to PTU reduced the uptake to 11% and of KI (1 mg./10 Gm.) to less than 10%, but did not further increase MIT/DIT. The addition of TH to PTU returned the total gland iodine from 4.0 μg. towards (7.2) normal (9.0), but did not change MIT/DIT. The addition of thiocyanate to PTU also did not change the MIT/DIT.

The effect of PTU in increasing MIT/DIT and T_3/T_4 did not seem to be due, therefore, to a partial block of the binding of iodine, a low gland iodine, a high thyroid-stimulating hormone or a high intrathyroidal iodide. The evidence suggests that PTU blocked the conversion of MIT to DIT and that, secondarily to low DIT and high MIT, more T_3 was produced relative to T_4. This would result in higher radioiodine uptakes relative to thyroid hormone production in patients maintained on antithyroid drugs than in normal subjects. Such patients, though making less total hormone, may make more triiodothyronine relative to thyroxine.

Effect of Parathyroidectomy on the Early Distribution of Radiocalcium in Rats

By *Lawrence G. Raisz and David F. Hammack.* Radioisotope Service, V. A. Hospital, and Department of Medicine, State University of New York, Upstate Medical Center, Syracuse, New York.

Tracer experiments with radiocalcium have demonstrated that a fraction of bone calcium exchanges rapidly with extracellular fluid calcium. In the present study the effect of parathyroid hormone on the magnitude of this fraction was assessed by measuring the distribution of a tracer dose of Ca^{45} 6 hours after intraperitoneal injection. Experiments were performed in parathyroidectomized (PTX) rats or in sham-operated controls either 24 hours or one week after operation. Exchangeable calcium (Ca_e) was estimated from the amount of Ca^{45} in the tissue and plasma specific activity. Young growing rats (80 days) were studied both at 24 hours and one week after PTX. Plasma calcium was reduced from 10 to 6 mg. % by PTX in both groups. At 24 hours, Ca_e was 79 mg. in sham-operated rats and 65 mg. in PTX animals. There was a significantly greater decrease in Ca_e one week after PTX (83 to 53 mg.). In old rats (300 days) studied one week after PTX, plasma calcium had decreased from 10.5 to 7.8 mg. %. Ca_e was only 40 mg. in sham-operated controls and did not decrease after PTX. These data indicate that Ca_e can decrease with age despite an increase in bone mass, and that parathyroid hormone can affect plasma calcium concentration independently of any change in Ca_e. In young, growing animals, the larger control values of Ca_e may be due to the high rate of new bone formation, and the progressive decrease after PTX could be ascribable to the following sequence of events: decreased parathyroid hormone→ decreased bone resorption→ decreased new bone formation→ decreased exchangeable calcium.

Metabolic Observations in Adult Hypophosphatasia

By *William R. Beisel, Frank K. Austen and E. G. Herndon, Jr.* Department of Metabolism, Division of Medicine, Walter Reed Army Institute of Research, and Department of Medicine, Walter Reed Army Hospital, Washington, D. C.

Metabolic balance studies of calcium, phosphorus and nitrogen have not been previously reported in patients with hypophosphatasia. An unequivical diagnosis of hypophosphatasia was established in a 36-year-old white male who had an unhealed femoral fracture of 18 months' duration, repeated serum alkaline phosphatase determinations averaging 0.9 S.J.R. units (±0.1 S.E. of the mean), absent white blood cell alkaline phosphatase and a urinary phosphorylethanolamine excretion of 10 mg./day.

Two balance studies were carried out using a solid diet (identical each day) containing 1.06 Gm. calcium, 1.30 Gm. phosphorus and 15.8 Gm. nitrogen. After equilibration and 18 days of control collections, the patient received metacortin during 3 successive 12-day periods in doses of 15, 30 and 60 mg./day. This was followed by 12 post-control days. In the second study the patient received 15 mg. of stilbesterol per day for 18 days.

Throughout the entire first balance study, the patient retained bone minerals with a cumulative positive calcium balance of 15.8 Gm. and a cumulative positive phosphorus balance of 14.6 Gm. Metacortin did not appreciably alter the rate of accretion of bone minerals at any dose, although a strikingly negative nitrogen balance occurred. At a dose level of 30 mg./day, 17 Gm. of nitrogen were lost in 12 days, and 40 Gm. of nitrogen were lost over a like period when 60 mg./day of metacortin were given.

At no time during metacortin or stilbesterol therapy did the serum alkaline phosphatase rise to within the normal range, and neither drug led to the appearance of stainable granules of alkaline phosphatase in the peripheral white blood cells. Nevertheless, during the 10 months the patient was studied, the fracture site demonstrated radiographic evidence of spontaneous healing.

The Effects of Exercise Upon Glucose Utilization in Man

By *Charles A. Sanders, Gilbert E. Levinson, Walter H. Abelmann and Norbert Freinkel.* Thorndike Memorial Laboratory, Second and Fourth (Harvard) Medical Services, Boston City Hospital; Department of Medicine, Harvard Medical School, Boston; and Howard Hughes Medical Institute.

Although there is ample evidence for a diminution of insulin requirements during muscular exercise, the mechanisms are not understood. Experiments were designed to minimize some of the difficulties in interpreting previous efforts.

Blood sugar of recumbent, fasting, normal human subjects was maintained at 120–150 mg.% by continuous infusion. The sustained hyperglycemia was instituted to (a) stop hepatic glucose output; (b) mobilize endogenous insulin; and (c) enhance the analytical accuracy of measurements of arterio-venous (A-V) glucose differences. At the end of 2 hours of stabilization of blood sugar, leg exercises were instituted for 30 minutes with a bicycle ergometer. Simultaneous blood samples from the brachial artery and from the cephalic, femoral and hepatic veins were obtained before, during and after exercise. The measured A-V differences for glucose afforded some index of glucose extraction in exercised (i.e., leg) and resting (i.e., arm) muscle beds, as well as in the splanchnic area. In 5 normal subjects, arterial blood sugar fell concomitant with unchanged or slightly decreased A-V glucose in the arm and the splanchnic area. However, late in exercise and for 5–10 minutes in the post-exercise period, A-V glucose differences in the leg were increased 3 to 4 fold. In two juvenile diabetics in whom insulin had been withheld for 24 hours and fasting blood sugar ranged from 150–280 mg.%, comparable studies without infusion of glucose disclosed enhanced glucose extraction in the exercised extremity with negligible blood sugar changes in other areas. The studies would indicate that exercise disparately promotes glucose extraction in exercised muscle and that this phenomenon is not acutely mediated by insulin, although a permissive or conditioning role of insulin cannot be excluded.

Effect of Glucagon on Homeostatic Balance between Hepatic and Peripheral Carbohydrate Metabolism

By *Dorothy H. Henneman and William Shoemaker.* Seton Hall School of Medicine, Jersey City, New Jersey, and Peter Bent Brigham Hospital and Harvard Medical School, Boston.

Intermediary carbohydrate metabolism of liver, splanchnic smooth muscle and hind limbs was studied in 15 normal, conscious dogs. Indwelling catheters were placed in crucial vessels prior to study, and hepatic and peripheral blood flow were measured according to the technics developed by Shoemaker. Concentration gradients

of glucose and lactic, pyruvic and citric acids were obtained.

Resting liver produced glucose (57.3 mg./min.) and cleared pyruvic, lactic and citric acids (2.4, 6.7 and 3.1 mg./min., respectively). Splanchnic blood flow was not measured; concentration gradients indicated minor glucose utilization and organic acid production. Skeletal muscle and bone utilized glucose (16 mg./min.) and pyruvic acid (0.75 mg./min.) and produced lactic acid (3.8 mg./min.); there was no significant metabolism of citric acid.

Infusion of glucagon (0.4 mg.) produced within 20 minutes an increase in hepatic (609 to 1660 ml./min.) and a decrease in peripheral blood flow (322 to 179 ml./min.). Hepatic glucose production increased rapidly to 498 mg./min., while hind limb utilization rose to 59 mg./min.; splanchnic utilization also increased. Hepatic clearance of pyruvic and lactic acids reversed temporarily to production 10 minutes after glucagon; thereafter, clearance of these progressively increased. Splanchnic production of organic acids increased. Peripheral utilization of pyruvic acid increased, rising to 1.1 mg./min. within 10 minutes; lactic and citric acid production progressively increased.

In starved dogs, glucagon produced minimal changes in hepatic and peripheral blood flow and diminished increases in hepatic glycogenolysis and in peripheral utilization of glucose and pyruvic acid. However, peripheral production of lactic and citric acids increased above normal, as did hepatic clearance of organic acids.

There appears to be a physiologic balance between hepatic glycogenolysis and peripheral production of carbohydrate metabolites which the liver then polymerizes for glycogen resynthesis. Glucagon initiates this homeostatic mechanism by producing immediate and simultaneous peripheral as well as hepatic metabolic and hemodynamic changes.

Differential Resin Binding of Insulin in Serum

By *Marvin L. Mitchell, William O. Whitehead and Mary E. O'Rourke.* Medical Service, Lemuel Shattuck Hospital, Boston, and Department of Medicine, Tufts University School of Medicine.

Alterations in serum-insulin binding have been demonstrated previously by electrophoretic and immunologic technics. A different approach to hormone binding has been provided with a simple in vitro system which quantitated the differential binding of labelled insulin from sera by a resin.

Sera containing insulin, labelled with I^{131}, were equilibrated for 1.5 hours in glass tubes with a measured volume of Amberlite IRA-400 anion exchange resin. Following equilibration, the tubes were counted in a conventional well-type scintillation detector, and the resin was then washed to remove the serum-bound radioactivity. The activity remaining on the resin divided by the initial activity measured the fraction of hormone bound by the resin ("resin index"). Thus, a low resin index reflected increased binding of hormone by serum and vice versa. Duplicate resin determinations were made on sera from 40 insulin-treated diabetics, 11 non-insulin–treated diabetics and 50 normal subjects. The mean difference between duplicate resin indices for all determinations was 1.2 ± 1.0% (1 S.D.).

Increased binding of insulin-I^{131} by the sera from previously treated diabetics was clearly demonstrated by a decreased resin index (12.0 ± 5.6%) when compared with the indices of normal controls (26.0 ± 2.6% $P < 0.001$) and non-insulin–treated diabetics (23.4 ± 3.6%, $P < 0.001$). Although the degree of insulin binding was apparently unrelated to maximum duration or dose of insulin, a minimal period of therapy was necessary before binding was detected.

Further differences between insulin–treated and control sera were shown when the addition of 0.01 to 1.0 units of stable insulin/ml. of serum produced increments in the diabetic resin indices but were without effect upon the control sera.

In summary, quantitative alterations in serum binding of insulin have been demonstrated in treated diabetes using the differential adsorption of insulin by a resin.

Clinical Experience with Chlorpropamide, A New Hypoglycemic Sulfonylurea Derivative

By *Franz Jost, Peter Masley, Richard J. Kennedy and William J. Grace.* Department of Medicine, St. Vincent's Hospital, New York, and New York University–Bellevue Medical Center.

Chlorpropamide, N-Propyl-N^1 (p-chlorobenzenesulfonyl) urea, a new hypoglycemic agent, has been used clinically in 30 diabetics of varying severity, ranging in age from 35 to 80. The drug is a potent hypoglycemic agent showing an effect on glycemia and glycosuria in all pa-

tients who received sufficient dosage. The initial dose was 1–2 Gm., while the maintenance dosage varied from 125 mg. to 1 Gm. depending on the severity of the diabetes.

When given in large doses, the drug has a prompt hypoglycemic action which is manifested within 24 hrs. In smaller doses there is a cumulative effect. The drug is excreted slowly so that its hypoglycemic effect is still apparent 3 to 5 days after stopping. Of the 30 patients there were 3 obvious failures, all of whom had been on 30 or more units of a long-acting insulin for 15 or more years. There were 12 patients who had never been treated, while the remainder had received some form of therapy, either insulin or tolbutamide. Patients who have used 10 to 70 units of insulin are now adequately controlled on chlorpropamide.

The most common untoward effect noted was hypoglycemia. Two patients had to be withdrawn from the study because of this, while in the others, divided maintenance dosage in small quantities was required to prevent the early symptoms of hypoglycemia. Peptic ulcer complaints developed in one patient. There was one case of dermatitis medicamentosa with leukopenia in a patient who manifested a similar syndrome 3 weeks earlier while on penicillin therapy. The most severe toxic reaction noted to date is a thrombocytopenia which responded rapidly to prednisone therapy.

These studies would indicate that chlorpropamide is a potent hypoglycemic agent. It would appear to be more effective than tolbutamide. It is well tolerated, its action is cumulative and its excretion is slow. With respect to tolbutamide, much smaller maintenance dosage is required. It is a relatively safe drug, but close surveillance, especially during the early period of administration, is indicated. No renal or hepatic toxicity has been noted.

Simple Laboratory Procedures in the Diagnosis of Cushing's Syndrome

By *John F. Maher, E. Garland Herndon and Laurence H. Kyle.* Department of Medicine, Georgetown University School of Medicine; Georgetown University Hospital; and Department of Metabolism, Walter Reed Army Institute of Research.

Twenty personally observed cases of Cushing's syndrome were studied to determine the value of simple laboratory studies as an adjunct to the diagnosis. Histologic diagnosis, accomplished in 18 of the cases, revealed 12 with hyperplasia, 1 with hyperplasia and adenoma formation, 4 with adenoma, and 1 with carcinoma. All laboratory procedures were done before adrenal stimulation or suppression tests were conducted. Using strict criteria and averaging at least 3 determinations of each test, 2 or more hematologic abnormalities were found in 70% of the patients. Polymorphonuclear leukocytosis was present in 50% of the cases, often in the absence of significant elevation of the total leukocyte count. As determined by direct counting, 10 of the 20 cases had intense eosinopenia, and as judged by a hematocrit elevation of at least 5% above normal, 25% of the cases showed polycythemia. With less rigid criteria, hematologic abnormalities were seen in all cases.

Moderate or marked osteoporosis occurred in 12 of the 20 cases as judged by roentgenograms of the lateral lumbar spine. In a similar number, resting blood pressure was elevated above 160 systolic or 110 diastolic. Overt diabetes or, more often, a clear-cut diabetic glucose tolerance test was seen in 70% of the patients, some of whom showed resistance to insulin. All patients demonstrated at least one of these abnormalities. Of the 6 possible abnormalities, 2 or more were present in 19 patients, and 3 abnormalities were apparent in the average case. While most suspicious cases deserve full hormonal evaluation, it is believed that simpler laboratory tests can aid considerably in strengthening or weakening the original diagnostic impression in the patient suspected of having Cushing's syndrome.

Steroid Excretion in Cushing's Syndrome and the Adrenogenital Syndrome

By *Herman E. Carr, Jr., William J. Reddy, Don H. Nelson and George W. Thorn.* Departments of Medicine, Harvard Medical School, and Peter Bent Brigham Hospital.

Steroid excretion, before and during ACTH stimulation, has been investigated in 10 women with Cushing's syndrome and in 1 woman with adrenogenital syndrome. These data are interpreted with reference to the findings in a group of normal female subjects. Cushing's syndrome was due to adrenocortical hyperplasia in 5 cases, adrenocortical adenoma in 3 cases and adrenocortical carcinoma in 2 cases. The single case of adrenogenital syndrome was attributable to

an adrenocortical adenoma. The biochemical parameters studied include total urinary 17-hydroxycorticoids (17-OH's), total urinary 17-ketosteroids (17-KS's) and fractionation of the major components of the 17-ketosteroids. The latter is accomplished by elution chromatography and resolves an 11-desoxy and an 11-oxy fraction. The desoxy fraction is further separated by means of the Pettenkoffer reaction as a measure of dehydroepiandrosterone (DHEA). The remaining major steroids in this fraction have been found to be androsterone and etiocholanolone (A + E). The 11-oxy fraction is not further resolved. This fraction in normals represents in large part in vivo conversion of cortisol to 17-ketosteroids.

On the basis of these studies, it may be possible to differentiate these individual disease entities.

Patients with Cushing's hyperplasia demonstrated moderate elevations in basal 17-OH excretion (12.3 mg./24 hr.) and in basal 17-KS excretion (18.9 mg./24 hr.), both of which were hyper-responsive to ACTH. 17-KS fractionation disclosed a slight increase in the 11-oxy fraction (28% of total as compared to 18% for the control group). Patients with Cushing's adenoma showed an elevated basal 17-OH excretion (16.1 mg./24 hr.) but a normal 17-KS basal excretion. This dissociation was maintained during ACTH stimulation in 2 patients with increased 17-OH response. In the third patient the 17-OH response was normal. In all 3 patients the 17-KS's responded normally. Fractionation revealed a markedly elevated 11-oxy fraction in these patients; basal values were 55% of the total, increasing to 64% on the second day of ACTH. Patients with adrenocortical carcinoma could be distinguished by marked elevation in both 17-OH and 17-KS excretion and by their non-responsiveness to ACTH. The average 17-OH value in 2 patients was 68.2 mg./24 hr. and 156 mg./24 hr. for the 17-KS. Fractionation showed a variable pattern in these patients, but there was a tendency for the most marked elevation to exist in the 11-desoxy ketosteroids.

The patient with adrenogenital syndrome had normal 17-OH's and total 17-KS's increased to 332 mg./24 hr. There was an equivocal response to ACTH. The major aberration, on fractionation, was found in the DHEA fraction.

Primary Aldosteronism, Observation on Two Cases

By *Felix M. Cortes, Charles R. Shuman, Bertram J. Channick and Marvin Lubart.* Temple University Medical Center, Philadelphia.

Two patients with primary aldosteronism due to aldosteronoma have been encountered during the past year. The clinical features manifested by these patients were entirely dissimilar and did not coincide with the syndrome described in many of the published cases. The first patient, a 45-year-old Negress under treatment for hypertension for 5 years at another hospital, was admitted in a state of peripheral circulatory collapse following an acute episode of diarrhea. Hypokalemia, attributed initially to diarrhea, proved resistant to therapy with potassium administration over a period of several weeks. She disclaimed any symptoms of weakness, paralysis or tetany prior to admission. The second patient, a 50-year-old white female, was admitted for treatment of menorrhagia. Hypertension and edema had been under treatment for 4 years. On 2 occasions, the administration of diuretics for treatment of edema had resulted in brief episodes of extreme muscular weakness. In the latter case, it was noted that the urine was alkaline in reaction with a specific gravity of 1.010. An electrocardiogram disclosed findings characteristic of potassium depletion. Serum electrolyte determination revealed hypokalemic alkalosis. In both instances, retroperitoneal CO_2 insufflation demonstrated an abnormal enlargement of the left adrenal gland. Twenty-four hour urine collection revealed abnormal aldosterone levels (80 μg. and 24 μg., respectively) with normal 17-ketosteroid and 11-oxysteroid levels. Surgical removal of the adrenals in both cases revealed almost identical adenomas. Large amounts of potassium salts were required to maintain normal serum levels of this electrolyte during the operative period. Postoperatively, the blood pressure declined to shock levels in one case and to normal in the other; in both, there was a transient period of hypertension during cortisone administration after operation. Abnormal glucose tolerance tests returned to normal after recovery. Both patients are now normotensive and remain in excellent health.

A Correlation of Plasma and Urinary 17-Hydroxy-Corticosteroid Levels during ACTH Stimulation

By *James Blair and J. C. Beck.* Royal Victoria Hospital, Montreal, Canada.

The adrenocortical response, as reflected in

the levels of free plasma and total urinary 17-hydroxycorticosteroids, to ACTH, administered at intervals of 6 hours for 48 hours, has been determined in 147 tests on 128 subjects. Included in the group were normal controls and patients with adrenocortical hyperfunction, obesity, adrenocortical hypofunction, hypothyroidism and miscellaneous illnesses. Usually, there is good correlation of the plasma and urinary levels. In many of the patients with adrenocortical hyperfunction or obesity, the degree of change, following stimulation, in the plasma levels is proportionally less than in that of the urine. The control levels prior to stimulation are generally elevated in the cases of adrenocortical hyperfunction. In a few of these patients there is a fall in the plasma levels following the first 4 hours of ACTH administration, despite an increase in the urinary levels. The plasma levels seem to be influenced by a greater number of variable factors than those of the urine. Preliminary studies have shown wide variation of the renal handling of the 17-hydroxycorticoids during ACTH stimulation, as well as smaller variations in plasma protein binding.

Isolation of Cortisol from a Pheochromocytoma

By *Patrick J. Mulrow and George L. Cohn.* V. A. Hospital, West Haven, Connecticut, and Department of Medicine, Yale University School of Medicine.

Tissue from a pheochromocytoma released a compound in vitro which was similar to cortisol.

The 169 Gm. adrenal tumor was surgically removed from a 62-year-old normotensive housewife. Histologic sections were compatible with the diagnosis of pheochromocytoma. Large amounts of catechol amines (more than 3 mg./Gm. of tissue) were extracted from the tumor. Postoperatively, the patient developed marked hypotension.

Slices of tumor were incubated in Kreb's Ringer bicarbonate buffer in an atmosphere of 95% O_2, 5% CO_2 for 3 hours at 37 C. An alkaline-washed chloroform extract of the media was evaporated in vacuo and the dried extract subjected to paper chromatography. An ultra-violet absorbing compound with a mobility similar to cortisol was detected in the toluene-propylene glycol system. Rechromatography of this substance in the Bush C system again revealed a compound with mobility similar to standard cortisol. This compound absorbed ultra-violet light, fluoresced in alkali and gave a Porter–Silber reaction.

Additional studies were carried out with frozen tumor tissue which was thawed and incubated in media containing TPN, glucose-6-phosphate and progesterone-16H^3. The media were extracted and chromatographed as described previously. Again, an ultra-violet absorbing compound with mobility similar to cortisol in 2 chromatography systems was detected. Following acetylation, this compound had the same chromatographic mobility as standard cortisol acetate and contained a small amount of radioactivity.

These results indicate that histologic criteria alone may be inadequate to describe the functional pathology of adrenal tumors. They also suggest that the stormy postoperative course which follows removal of a pheochromocytoma may be in part due to the removal of corticosteroid-producing tumor.

Observations on the Uricosuric Effects of Zoxazolamine in Gouty Subjects

By *Thomas B. Connor, T. Nelson Carey, Thomas Davis and Harris Lovice.* University of Maryland, School of Medicine, Department of Medicine, Baltimore.

Zoxazolamine (2-amino-5-chlorobenzoxazole) (Flexin) has been used widely as a skeletal muscle relaxant. In 1957, one of the authors (T.N.C.) observed that this agent also produced a marked lowering of the serum uric acid in man. Accordingly, an investigation of the uricosuric properties and therapeutic value of the compound in gouty subjects was undertaken.

Six patients with intercritical gout and serum urate levels of 9 to 12 mg.% were each given a single 1.0 Gm. oral dose of zoxazolamine. In each subject there occurred a marked rise in urinary urate excretion that reached a peak 4 to 6 fold increase within 90 to 180 minutes. Serum urate levels decreased 1.5 to 3.0 mg.% during this same time period. Coincident renal clearance studies were performed and revealed a 4 to 8 fold increase in ^{c}urate/^{c}creatinine in each patient. ^{c}Creatinine was not significantly affected in any study period.

Three of these subjects were placed on constant diets for 18 to 24-day periods and given zoxazolamine, 0.25 Gm. 4 times daily. In each instance, a sustained increase (25% or more) in 24-hour urinary urate excretion above control values was observed, and serum urate concentration fell to normal levels within 48 to 72 hours.

Cessation of zoxazolamine therapy resulted in a return of serum and urine urate values to pretreatment levels within 48 hours. Following another suitable control period, zoxazolamine was resumed in the same dosage, and in each patient an identical uricosuric and hypouricemic effect was obtained.

In one patient, the uricosuric effects of 1.0 Gm. oral doses of probenecid and zoxazolamine were compared. Measurement of ^{C}urate/^{C}creatinine and 24-hour urine urate excretions during 6-day periods of therapy with each drug revealed a consistently greater uricosuric effect from zoxazolamine.

Four of these patients have been maintained on oral zoxazolamine therapy in 1.0 Gm. doses daily for periods of 2 to 9 months. The compound appears to have definite value in the management of tophaceous gout in these individuals, as evident from the following observations: (1) Disappearance of tophi from the ears in one patient. (2) Maintenance of serum urate levels below 7.0 mg.% as long as therapy is continued. In one patient the dose had to be increased to 2.0 Gm. daily to maintain this effect. (3) Definite decrease in frequency of acute attacks of gout. (4) Marked reduction in chronic joint pains in between acute attacks. (5) Consistent maintenance of uricosuric effect on periodic 24-hour urinary urate determinations.

Untoward reactions to zoxazolamine therapy were not observed in these patients.

Changes in Blood and Tissue Electrolytes during the Acute and Early Adaptive Phases of Exposure to CO_2

By *George Nichols, Jr.* Departments of Medicine and Biological Chemistry, Harvard Medical School.

Marked increases in plasma, muscle and brain bicarbonate and a fall of pH in these body compartments has recently been reported by us in rats exposed to 24% CO_2 in air. These findings suggested that the concentrations or total amounts of the other electrolytes and water of these tissues might have changed as well as the total CO_2. Therefore, samples of plasma, muscle, brain and bone were obtained from the animals previously reported and analyzed for water, Na, K and Cl. In addition, total PO_4 of brain, muscle and bone and bone calcium were measured. In addition to the controls, samples from animals exposed to CO_2 for ½, 1, 3, 5, 7, 15, 24 and 48 hours were examined. The intracellular composition of muscle and brain was calculated from the plasma values using a chloride space.

Plasma chloride fell slowly as the exposure period lengthened, and the plasma bicarbonate increased, but the sodium concentration did not change. No change in plasma water occurred until 48 hours, when there was a slight decrease. A slight increase in the mean plasma potassium concentration occurred, but this change was not seen in all animals. These changes in the "fixed" electrolytes of the plasma were in the direction of metabolic alkalosis which partly compensated for the respiratory acidosis.

In muscle no such consistent pattern occurred. At ½ hour of exposure the Cl space increased almost 50%, while intracellular K and PO_4 increased also. By 1 hour, K and the chloride space were normal and phosphate was low. By 48 hours, both K and PO_4 in the cells were elevated and the chloride space was increased again about 25%. Intracellular sodium decreased in ½ hour and remained low. Thus the compensation of the increased HCO_3^- and H^+ ion concentrations in these cells seemed due to changes in phosphate, sodium and distribution of water. In brain, a fall in K concentration was the only consistent change. Sodium and phosphate fluctuated but showed no sustained trend. In contrast to the muscle the chloride space decreased in this tissue. Finally, no consistent change in bone composition occurred.

From these data it is concluded that while the changes in the plasma electrolyte concentrations follow a definite pattern consistent with the development of a compensatory metabolic alkalosis, the response of the tissue electrolytes to respiratory acidosis varies both with the type of tissue and the duration of exposure.

Acid Excretion in Rubidium- and Cesium-substituted Rats

By *Philip W. Hall, III and Arnold S. Relman.* Department of Medicine, Boston University School of Medicine, and Evans Memorial Department of Clinical Research and Preventive Medicine, Massachusetts Memorial Hospitals, Boston.

Previous studies have shown that rubidium and cesium can replace a major part of intracellular cation in vivo and will cure the extracellular alklosis of K-depletion in rats, probably by displacement of intracellular hydrogen. Ad-

ministration of rubidium chloride produces hyperchloremic metabolic acidosis, without any immediate increase in renal acid excretion.

The present experiments were designed to observe the basal excretion of acid as well as the response to an acid load in rats whose body and renal tissue potassium had previously been partially replaced by rubidium or cesium, and to compare their behavior with that of untreated K-depleted animals and normal controls. Acid excretion was studied in one half of each group of animals for a 24-hour period while they were receiving intraperitoneal ammonium chloride, 1 mEq./100 Gm. body weight, and in the other half while they were receiving an equivalent amount of sodium chloride. During the study period and for approximately 12 hours before, the animals were allowed only glucose and water.

During sodium chloride loading, ammonium excretion was high, as expected, in the K-deficient alkalotic animals but not increased in the rats previously treated with rubidium or cesium. Rubidium rats, however, had a significant degree of acidosis, but the cesium rats did not. Glutaminase I activity was increased in the K-deficient animals as well as in the cesium- and rubidium-substituted animals. All groups were able to excrete virtually all of the acid load within the period of study, due mainly to increments in ammonia. However, the cesium- and rubidium-substituted animals had little or no change in urine pH, whereas the normal and K-depleted groups did.

The failure of rats to increase ammonium excretion in response to rubidium-induced extracellular acidosis may be explained by a normal or possibly increased renal intracellular pH, just as increased ammonium excretion in K-deficient alkalotic rats may be due to decreased intracellular pH. Administration of ammonium chloride probably lowers intracellular pH and so results in a normal response in all groups. Extracellular pH, urine pH and glutaminase I activity, on the other hand, do not correlate with ammonium excretion in these experiments.

Studies on the Biosynthesis of Monounsaturated Fatty Acids

By *D. K. Bloomfield and Konrad Bloch.* Converse Memorial Laboratory, Harvard University, Cambridge, Massachusetts.

The metabolic and possible therapeutic role of unsaturated fatty acids has recently received increased interest. It seemed worthwhile, therefore, to study the biosynthesis of these acids. Previous work has shown that an unsaturated fatty acid (oleic, linoleic or linolenic) is required for the growth of yeast under strictly anaerobic conditions. It is implied by this observation that the synthesis of olefinic fatty acids in yeast is an aerobic process. We studied resting yeast under anaerobic conditions and found that they converted up to 10% of trace amounts of acetate 1-C^{14} to long chain fatty acids. Over 90% of this fatty acid was found to be saturated, and if the same yeast were washed free of acetate 1-C^{14} and reincubated in the presence of oxygen, over 50% of the saturated fatty acids were desaturated. Cell-free Hughes' Press extracts of yeast were then shown to desaturate stearic and palmitic acids 1-C^{14} to oleic and palmitoleic acids 1-C^{14}, respectively. Centrifugal fractionation of the cell-free extract showed that both a soluble and a particulate fraction were necessary for enzymic desaturating activity. It has not been possible to solublize the particulate activity. The enzyme is very sensitive to pH changes. Activity is optimal at pH 6.6 and zero at pH 7.4 and above. The soluble fraction is completely inactivated upon dialysis but regains activity on addition of TPNH or a TPNH-generating system and divalent cation. TPN, DPN, DPNH or a DPNH-generating system were also active, but less so than TPNH. No activity has been found to date in rat liver homogenates. The oxygen requirement appears to be absolute. Oxygen cannot be replaced by electron acceptors. The process is stimulated by cyanide and inhibited by cytochrome C. All of these are properties typical of oxygenase reactions involving oxygen and TPNH.

GASTROINTESTINAL SYSTEM

Osmolar Clearance as a Basic Mechanism of Gastric Electrolyte Secretion

By *Basil I. Hirschowitz.* Fels Research Institute, Temple University School of Medicine, Philadelphia.

In an attempt to define gastric secretion in terms of some basic mechanism, the osmolar clearance was determined in 12 hospital patients free of peptic ulceration. In each, four 15-minute samples were aspirated in the basal state and a further 5 after the subcutaneous injection of 0.04 mg. histamine acid phosphate/Kg. body weight. Measurements were made of gastric juice volume (V), and the concentrations therein of hydrogen ion (H), chloride (Cl_g), sodium (Na_g), potassium (K_g) and osmolality (O_g) in each sample. Blood was drawn before and after the test and the plasma assayed for osmolality (O_p), sodium (Na_p), potassium (K_p), chloride (Cl_p) and bicarbonate (HCO_{3p}).

Under both stimulated and unstimulated conditions the osmolar clearance $\frac{V.O_g}{O_p}$ (or C_{os}) was used to calculate the sodium "load": $\frac{V.O_g}{O_p} \times Na_p$ (or C^{os}_{Na}) in mEq./15 minutes. This figure showed a persistent excess over the amount of acid secreted (V.H); this excess was found to equal almost exactly the output of sodium by the stomach ($V.Na_g$). Thus $\frac{V.O_g}{O_p} \times Na_p = V(H + Na_g)$ with an error of ± 0.4%. The relationship held from an acid output of 0.2 to 17.0 mEq. per 15 minutes, indicating a 1:1 relationship between the sodium load and the secretion of acid plus sodium by the stomach.

During maximal secretion, O_g may equal O_p, and the formula $V(H + Na_g) = \frac{V.O_g}{O_p} \times Na_p$ reduces to $H + Na_g = Na_p$. This suggests that the concentration of H ion in gastric secretion is unlikely to exceed the concentration of plasma sodium and, furthermore, that it is derived from plasma sodium by a 1 for 1 exchange within the framework of an osmolar clearance mechanism.

In rats, alterations of plasma osmolality by the injection of hyper and hypotonic NaCl solutions did not alter significantly this relationship between C^{os}_{Na} and $(H + Na_g)$.

The Effect of Serotonin on Human Gastric and Pancreatic Secretion and Bile Flow

By *Richard R. Pichel Warner, Henry D. Janowitz and David A. Dreiling.* Departments of Medicine and Surgery, Mount Sinai Hospital, New York City.

Since animal studies have shown serotonin to be an inhibitor of gastric acid secretion and preliminary studies have suggested a similar effect in man, investigation of the secretory response to serotonin by the stomach and pancreas in the basal state, as well as bile flow, was undertaken in man.

Gastric and duodenal contents in fasting individuals, continuously and separately aspirated by means of a double lumen tube, were pooled in 3 or 4 successive 20-minute periods, following which serotonin was administered i.v. at the rate of 10 μg./Kg./min. for one hour. During the administration of serotonin, the gastric and duodenal aspirates were each again collected in 3 or 4 pooled 20-minute periods.

In response to i.v. serotonin, 11 individuals exhibited a slight increase in volume of gastric juice (average increase of 14%), and 7 individuals having free acid prior to serotonin exhibited a definite decrease in free acid (average decrease of 38%) during administration of the drug. In 8 individuals having measurable total acid prior to serotonin, a decrease averaging 47% in total acid occurred during the administration of the drug.

The volume of the duodenal aspirate exhibited no significant change during i.v. serotonin in 10 cases studied. In 7 cases in whom bicarbonate concentration was determined, and in 8 in whom amylase concentration was determined, no significant change occurred. In 9 cases, the bile pigment flow (quantitated as cc. duodenal aspirate x icterus index) decreased by an average of 21% during i.v. serotonin.

It is concluded that i.v. serotonin in the dosage given: (1) partially inhibits the secretion of acid by the human stomach and concurrently effects a slight increase in total volume of gastric secretion, possibly by way of its known mucus-stimulating properties; (2) has no significant effect on external pancreatic secretion; and (3) partially inhibits bile pigment flow.

Localization of the Effect of Intrinsic Factor

in the Rat Small Intestine in Vitro

By *Victor Herbert, Zaida Castro and Louis R. Wasserman.* Department of Hematology, Mount Sinai Hospital, New York City.

This study was undertaken to determine if the effect of intrinsic factor on vitamin B_{12} uptake in various portions of the rat small intestine is similar in vitro and in vivo.

The small intestine of each rat was removed, everted, cut into 5 cm. lengths and sealed. The sacs were incubated at 3 C. for one hour in Krebs-Ringer-Tris (KRT) pH 7.5 buffer, in air, with 1 ml. 0.9% NaCl added. The added NaCl of even-numbered sacs contained 0.5 mg. of a hog intrinsic factor concentrate (HIFC). After washing thrice, the sacs were incubated a second hour in KRT containing 900 $\mu\mu$g. Co^{60}-B_{12}/ml. They were again washed and their retained radioactivity determined.

HIFC did not enhance the vitamin B_{12} uptake of duodenal sacs, somewhat enhanced that of jejunal sacs and markedly enhanced that of ileal sacs.

These results closely resemble those obtained by Reynell, Spray and Taylor who used living rats. In vivo, the intrinsic factor effect was not as marked in the fourth quarter of the small intestine as it is in vitro, probably because Co^{60}-B_{12} is given *per os* to the living rat and its concentration in the terminal quarter of the small intestine is reduced by the amount absorbed higher up. In vitro, the entire small intestine is exposed to the same concentration of Co^{60}-B_{12}.

These studies suggest that the everted sac system is valid for studying the mechanism of intrinsic factor action. They indicate that the failure of in vivo attempts to enhance vitamin B_{12} absorption of rats with HIHC may be due to phenomena other than intrinsic factor species specificity.

Factors Influencing the Absorption of Radioactive Sodium (Na^{24}) from the Jejunum

By *Victor W. Groisser and John T. Farrar.* Department of Medicine, Seton Hall College of Medicine, Jersey City, New Jersey; Medical Service, V. A. Hospital, New York City; and Department of Medicine, Cornell University Medical College, New York City.

The factors which influence the speed and efficiency of absorption from the human small intestine are not well understood. To provide insight into the mcehanisms involved in the mucosal transfer of more complex substances, several factors affecting the absorption of a simple ion, sodium, have been investigated.

The intestinal absorption of radioactive sodium has been studied by the method of Scholer and Code 46 times in 23 convalescent hospital patients. Na^{24} was administered into the jejunum by means of a tube, and simultaneously Na^{22} was given intravenously. Arterial samples were drawn in rapid succession, and integration of the Na^{22} and Na^{24} curves permitted calculation of the total net absorption of Na^{24}. Intrajejunal pressure fluctuations were recorded during the absorption period. Utilizing the initial curve as a control, the influence of the following factors on a second identical absorption test one hour later was observed: (1) intravenous Probanthine; (2) intravenous Probanthine (during the 1-minute period of Na^{24} administration the tube was withdrawn through approximately 2½ feet of jejunum); (3) no alteration of control conditions.

In 23 patients, the mean total absorption in 12 minutes was 91.6% of the administered dose. In 9 patients, a second identical test was performed under control conditions. The intraindividual differences in absorption were very significantly less than the interindividual variations within this group. No consistent relationship was noted between the jejunal motility and the rate of absorption in any of the patients. Probanthine administration consistently decreased Na^{24} absorption, and this decrease was not appreciably reversed by increasing the length of jejunum to which the isotope was exposed. The simple concentration curves in all tests strikingly parallel the more complex total absorption data.

These studies show that determination of a control absorption curve in each patient will permit more precise evaluation of factors which influence absorption.

An Experimental Malabsorption Syndrome Induced by Neomycin

By *Eugene D. Jacobson, Robert B. Chodos and William W. Faloon.* College of Medicine, Upstate Medical Center, and V. A. Hospital, Syracuse, New York.

The finding of steatorrhea during oral administration of neomycin in patients without intestinal disease has prompted further characterization of the effect of neomycin on intestinal absorption.

In 6 subjects, plasma carotene was determined during 1-week periods of control, neomycin sulfate administration (12 Gm. daily) and postneomycin control. Supplementary carotene was given daily throughout the study. Comparison studies were afforded in 4 subjects given cathartics. All 6 subjects given neomycin manifested rising plasma carotene during the first control period, a fall of 30% or more (of maximum control values) during the neomycin period and a post-withdrawal rise. Patients receiving magnesium sulfate showed no fall in plasma carotene. One subject given castor oil exhibited a drop less than that seen with neomycin.

In other studies, plasma Fe^{59} changes following oral administration, urinary Co^{60}-labelled B_{12} excretion (Schilling), urinary d-xylose excretion, glucose tolerance and serum cholesterol determinations were performed in different groups of patients before and after 3 to 7 days of neomycin.

When neomycin was given, Fe^{59} absorption was delayed or decreased in 4 of 6 subjects, and urinary B_{12} excretion was reduced by 20% to 60% of pretreatment levels in 4 of 6 patients. Urinary d-xylose excretion fell in 6 of 8 subjects by 35% or more; 3 of these manifesting neomycin effect were investigated at 1 or 2-week intervals after withdrawal of neomycin, and in 2 of this group d-xylose excretion returned to pretreatment levels. Four of 6 subjects showed lowered glucose tolerance curves, and in 3 of these the curves were flat in the treatment period. Serum cholesterol fell by 19% or more in 6 of 9 patients during neomycin administration.

These findings indicate that neomycin is capable of producing a wide spectrum of malabsorptive errors which resemble those noted in idiopathic steatorrhea.

Identification and Analysis of Nonglucuronide Conjugates of Bilirubin in Human Bile

By *Kurt J. Isselbacher and Elizabeth A. McCarthy.* Medical Service, Massachusetts General Hospital, and Department of Medicine, Harvard Medical School, Boston.

Our interest in hepatic conjugation mechanisms has prompted a study of the possible physiologic occurrence of nonglucuronide conjugates of bilirubin in the mammalian organism. Previous observations by Billing et al. and Schmid and Schachter have indicated that "direct-reacting" bilirubin is a glucuronide conjuate which is alkali-labile and in which the glucuronide linkage is at the carboxyl group of the bilirubin molecule. The fact that a definite fraction of bilirubin conjugates in bile are alkali stable and the knowledge that the liver often forms nonglucuronide conjugates in detoxification processes led us to suspect that other polar derivatives of bilirubin might occur.

Rats with bile fistulas were injected with S^{35}-labeled inorganic sulfate and the pigments purified and isolated. We have been able to demonstrate the existence of "direct-reacting" bilirubin conjugates labeled with $S^{35}O_4$ by means of paper chromatography in various solvent systems, radioautography and acid hydrolysis. Data obtained by the use of diazomethane and hydroxylamine indicate that the sulfate group is attached at the hydroxyl group of the bilirubin. As would be expected from theoretical considerations, this pigment is alkali stable and present data indicate that it constitutes approximately 15% of the total bilirubin conjugates of normal human bile.

Additional studies using β-glucuronidase hydrolysis also serve to emphasize the existence of nonglucuronide bilirubin conjugates, which in normal human bile samples amount to approximately 24%. From these data and observations on alkali stability, it is apparent that in addition to bilirubin glucuronide and bilirubin sulfate, a small amount (approximately 9%) of other carboxyl-linked, alkali-labile derivatives also occur. Bilirubin sulfate has been demonstrated also in the urine and serum of rats following bile duct ligation.

Enzymatic synthesis of a sulfate conjugate of bilirubin has been observed in vitro in liver homogenates. For this synthesis adenosine triphosphate is required. Presumably, the mechanism of sulfate conjugation involves the "active-sulfate" (adenosine-3'-phosphate-5'-phosphosulfate) described by Lipmann and Robbins.

Hepatic Transport of I^{131}-Diodrast

By *Paul I. Jagger, Jerome B. Block and Belton A. Burrows.* Robert Dawson Evans Memorial and Massachusetts Memorial Hospitals; Radioisotope and Medical Services, Boston V. A. Hospital; and Boston University School of Medicine, Boston.

Hepatic as well as renal uptake of I^{131}-Diodrast occurs at low plasma concentrations. Its transport into the bile after i. v. doses of 0.1 mg.

(20 μc.) has been studied in normal human subjects and in patients with liver disease. External monitoring and plasma sampling demonstrated that I^{131}-Diodrast may be flushed from the liver into the plasma with subsequent "carrier" doses of nonradioactive Diodrast. This flushing did not occur with Cholografin, Hypaque, para-amino hippurate, phenolsulfonphthalein, Brom-sulfalein or aminophylline.

Prior administration of carrier Diodrast (1.0 Gm.), alone among the compounds studied, markedly decreased hepatic uptake and biliary secretion of I^{131}-Diodrast in patients with common bile duct drainage. An inhibitory effect on the biliary secretion of I^{131}-Diodrast, after hepatic uptake had occurred, could be demonstrated with Cholografin, but not with the other compounds used.

With in vitro dialysis experiments, it was shown that the dosages of carrier Diodrast used did not prevent plasma protein binding of I^{131}-Diodrast. It would therefore appear that carrier Diodrast competes for hepatic cellular uptake with I^{131}-Diodrast, either displacing it from the liver cells or, if given previously, preventing its uptake. Cholografin appears to have a separate mechanism for hepatic uptake, but a subsequent excretory pathway in common with Diodrast. Prior to its transport into the bile by this common pathway, I^{131}-Diodrast apparently occupies specific hepatic uptake sites that are saturated with small doses of Diodrast.

Elevated Serum Glutamic Oxalacetic Acid Transaminase and Central Necrosis of the Liver

By *Thomas Killip, III and Mary Ann Payne*. Department of Medicine, New York Hospital–Cornell Medical Center.

Acute elevation of the serum glutamic oxalacetic acid-transaminase (SGO-T) activity to very high levels is occasionally encountered in patients with cardiovascular disease.

During a 30-month period, SGO-T greater than 500 u./ml./min. was found in 17 patients suffering from cardiac disorders without evidence of primary liver or gall bladder disease. Eleven had recent myocardial infarction; 6 had chronic heart failure.

In all 17 cardiacs the transaminase elevation was associated with hypotension or shock, often persisting for many hours. Fifteen had evidence of right heart failure. Eight of the 10 patients with SGO-T greater than 700 u./ml./min. died. Five of the 7 with levels 500–700 u./ml./min. survived. Seven of the 8 patients autopsied had extensive central necrosis of the liver; in one, the changes were less marked. Pathologic abnormalities in other organs were not consistent.

As a control 18 autopsied patients with cardiac disease who died within 30 hours of an SGO-T determination were reviewed. Eight had recent myocardial infarction; 8 died of heart failure. None of the 7 with normal SGO-T had central necrosis of the liver or hypotension, although 4 had severe right heart failure, thus suggesting that right heart failure alone is not sufficient to produce central necrosis. Five of the 10 patients with slight to moderate SGO-T elevation had slight to moderate central necrosis and hypotension. One patient with prolonged hypotension and severe central necrosis had an SGO-T of 408 u./ml./min. 30 hours prior to death.

Extensive central necrosis of the liver is associated with an acute rise of SGO-T to high levels. Prolonged hypotension appears to be an important factor in the pathogenesis of the hepatic necrosis. When SGO-T is utilized as an index of myocardial necrosis, the possibility that hepatic central necrosis is contributing to the abnormal values must be considered.

Serum β-Glucuronidase in Patients with Liver Disease

By *E. P. Pineda, J. A. Goldbarg, B. M. Banks and A. M. Rutenburg*. Yamins Surgical Research Laboratory, Beth Israel Hospital, and Department of Surgery, Harvard Medical School, Boston.

β-Glucuronidase activity was assayed in the sera of 230 patients with primary or secondary liver disease using a chromogenic substrate, 6-bromo-2-naphthyl-β-D-glucopyruronoside.

Patients with acute hepatitis showed elevated β-glucuronidase activity during the acute phase of the disease with return to normal on recovery. Patients who developed chronic hepatitis or a rapid fatal course showed depressed levels. Patients with mild and moderate cirrhosis had increased β-glucuronidase, whereas most of those with severe cirrhosis had depressed levels.

Fifty-five % of patients with obstructive jaundice due to stones or pancreatic cancer had elevated β-glucuronidase activity, whereas only 15% of the nonjaundiced group with these diseases had elevations. Increased activity was encountered in

patients with a protracted clinical course, prolonged jaundice or severe biliary obstruction. Relief of biliary obstruction resulted in the return of enzymatic activity to normal after 7–21 days.

In a control group of 455 patients, 88% of those with nonmalignant diseases and 83% with cancer of various organs without demonstrable hepatic metastases had normal β-glucuronidase levels. Most patients with increased glucuronidase levels also had abnormal conventional tests of liver function.

Histochemical assay for β-glucuronidase in liver biopsies obtained at operation from patients with obstructive jaundice or hepatitis also showed a high order of enzymatic activity, whereas glucuronidase in hepatic tumor metastases was depressed. The corresponding increase of glucuronidase activity in liver and serum suggests that the liver is a major site of origin or a control organ for changes in enzymatic activity observed in the blood.

Serum β-glucuronidase levels provided confirmatory evidence of hepatic dysfunction, and serial determinations were of value in following the clinical course of patients with liver disease as well as in predicting deterioration of hepatic function.

The Effect of Changing Serum Osmolality on the Release of Antidiuretic Hormone in Certain Patients with Decompensated Cirrhosis of the Liver and Low Serum Osmolality

By *Laurence E. Earley and Charles A. Sanders.* Second and Fourth (Harvard) Medical Services, Thorndike Memorial Laboratory, Boston City Hospital, and Department of Medicine, Harvard Medical School, Boston.

The patient with decompensated Laennec's cirrhosis often has a low serum sodium and osmolality. Such patients may excrete a concentrated urine in the presence of a lower than normal serum osmolality and also may undergo a water diuresis in response to water loading. Thus, it would appear that the release of antidiuretic hormone may be regulated about a lower than normal tonicity of the body fluid, or that its release is regulated by some stimulus other than tonicity. This study concerns patients with decompensated cirrhosis who have a lower than normal serum osmolality and who are able to excrete water loads. Our purpose was to determine if in such patients changes in body tonicity resulted in changes in the release of ADH, as reflected by alterations in the flow and concentration of the urine.

Patients with serum osmolalities of 272 mOsm. or lower were selected. All had ascites and jaundice without evidence of cardiovascular or renal disease. Water loads of about 1500 ml. were given intravenously and maintained. Six subjects responded with a water diuresis and were then given hypertonic sodium chloride in an amount calculated to restore the serum osmolality to its initial low level. In all 6 subjects, antidiuresis promptly ensued when the serum osmolality was returned to its initial level. During the water diuresis the serum osmolality was lowered by an average of 12 mOsm. less than the initial level. Minimal urine osmolalities were less than 60 mOsm. Immediately following the hypertonic saline, the serum osmolalities returned to within 3 mOsm. of the initial level, and maximal urine osmolalities during the antidiuresis ranged from 323 to 544 mOsm. There were no significant variations in the rate of solute excretion, and, where measured, the clearance of creatinine remained within normal limits. Total body volume was kept constant after the initial water load.

It is concluded that these subjects with low serum sodium and total solute concentrations were sensitive to further changes in tonicity, and that the release of ADH was regulated about a lower than normal level of osmolality.

Hyperventilation and Arterial Hypoxia in Cirrhosis of the Liver

By *H. O. Heinemann, C. Emirgil and J. P. Mijnssen.* Department of Medicine, Francis Delafield Hospital, and College of Physicians and Surgeons, Columbia University, New York City.

Hyperventilation causing respiratory alkalosis is known to occur in patients with cirrhosis of the liver. The mechanism maintaining hyperventilation remains obscure. The present study was undertaken to establish whether arterial hypoxemia, also known to be present in this disease, maintains hyperventilation in the face of normal pH and low arterial carbon dioxide tension.

To exclude intrinsic lung disease as a cause of hypoxemia, the lung volumes, maximal breathing capacity, minute ventilation, diffusing capacity for oxygen, arterial blood oxygen and carbon dioxide content and tensions were measured according to conventional methods.

The results show that patients with cirrhosis of the liver hyperventilate at rest in the absence

of increased oxygen consumption or intrinsic pulmonary disease. This leads to mild, compensated respiratory alkalosis as indicated by the low carbon dioxide tension and normal pH. The partial pressure for oxygen in arterial blood is low, despite elevated alveolar oxygen tension and normal diffusing capacity for oxygen. This seems to be best explained by increased admixture of venous blood via abnormal vascular communications between the portal and pulmonary vascular bed. The observed arterial oxygen tension is not of the order of magnitude commonly assumed to be necessary for stimulation of the respiratory center. The "primary" hyperventilation in cirrhosis of the liver remains therefore unexplained.

Combined Diuretic and Steroid Therapy in Cirrhosis with Ascites

By *Robert S. Morrison and Thomas C. Chalmers.* Medical Service, Lemuel Shattuck Hospital, Boston, and Department of Medicine, Harvard Medical School.

Currently available diuretics have disappointing effects in patients with cirrhosis and ascites. The present study evaluates the potentiation of mercuhydrin and chlorothiazide effects by the synthetic, "anti-aldosterone" steroid, spirolactone (3-[3-oxy-17 Beta-hydroxy-19-nor-4-androsten-17 Alpha-yl] propionic acid-Gamma-lactone, SC 8109) and by prednisone. Fourteen cirrhotic patients with baseline sodium excretions <1 mEq./day were studied while on a 200 mg. sodium diet. Sodium, potassium, chloride and creatinine were determined in the urine daily and in the blood every third day. Drugs were administered as follows: mercuhydrin, 2 cc. i. m. daily; chlorothiazide, 1 Gm. p. o. every 12 hours; spirolactone, 100 mg. p. o. or i. m. every 8 hours; and prednisone, 10 mg. p. o. every 8 hours.

When each drug was given alone on several occasions the following mean daily excretions of sodium were obtained: mercuhydrin, 24 mEq.; chlorothiazide, 17 mEq.; spirolactone, 16 mEq.; and prednisone, 2 mEq. Combinations of mercuhydrin with spirolactone or prednisone gave average outputs of 76 and 73 mEq., respectively, significantly greater than either agent alone in the same patients ($p<0.01$). In each of 2 patients chlorothiazide plus spirolactone or prednisone resulted in outputs averaging 160 and 80 mEq., respectively. Combination of spirolactone and prednisone in 4 patients resulted in mean outputs of 70 mEq. When mercuhydrin was given several times to each of 4 patients while they were receiving both spirolactone and prednisone, the mean output rose to 193 mEq. of sodium per day. The one patient given chlorothiazide plus both steroids excreted an average of 142 mEq. of sodium per day. After cessation of steroids, sodium output returned to the previous baseline of one mEq. per day.

It is concluded that the sodium retention in cirrhosis can be overcome by the combined approach of (1) suppressing adrenal cortical function with prednisone, (2) antagonizing aldosterone with spirolactone, and (3) diminishing tubular reabsorption of sodium by the addition of a mercurial diuretic or chlorothiazide.

Use of an Antialdosterone Compound for Fluid Retention in Hepatic Disease

By *O. Dhodanand Kowlessar, Bayard Clarkson and Marvin H. Sleisenger.* Department of Medicine, New York Hospital–Cornell Medical Center, New York City.

Oral administration of Spirolactone (SC-8109), 500 to 1,000 mg. per day for 4 to 16 days on 4 occasions in 2 patients with Laennec's cirrhosis, induced a significant diuresis in both individuals (6 lbs. and 23 lbs., respectively). These subjects with marked ascites and edema had become refractory to conventional therapy which included mercurials, chlorothiazide and acidifying agents, both individually and in combination.

Detailed studies of water and electrolyte balance revealed that the diuresis in these individuals accompanied a significant natruresis, with rise in sodium output from 3 to 68.2 mEq./24 hours and 20 to 190 mEq./24 hours in each patient, respectively. In one individual, there was a sharp drop in urinary sodium loss each time the agent was withdrawn. During the course of therapy, these patients were given 51.3 mEq. sodium/day in their diet in order to reduce endogenous aldosterone secretion to a minimum. This degree of sodium restriction per se had been without benefit.

Other significant findings in this study were: sodium to potassium ratio in saliva, measured in one subject, increased over fourfold during periods of SC-8109 treatment, indicating an antisteroidal effect. In addition to the fluid loss, which was moderate in one subject and marked in the other, there also appeared to be some improvement in liver function tests following diuresis.

Of special note are the facts that in another

patient given oral SC-8109, despite a rise of urinary sodium from 3.6 to 21.6 mEq./24 hours, no diuresis occurred in 3 days. In 2 other individuals with Laennec's cirrhosis and fluid retention, the material was ineffective when given intramuscularly, although in each instance urinary sodium rose appreciably. Failure to induce diuresis may be attributable to the short period of therapy in these patients, which was only 3 days in each instance.

Pleural Effusions in Hepatic Cirrhosis

By *Bonnie P. Malvea, Masafumi Seki and Thomas C. Chalmers.* Medical Service, Lemuel Shattuck Hospital, Boston, and Department of Medicine, Harvard Medical School.

Pleural effusion in cirrhosis of the liver has long been considered a medical oddity. An impression that its frequency has been increasing led to this study of its incidence and mechanism. Of 105 living patients, pleural effusion was found by x-ray in 11. Of 24 autopsied patients, 15 had more than 250 cc. of free pleural fluid on one or both sides. Seven had questionable nonhepatic causes of the effusion, and in 8 no other cause was apparent.

Five patients with both ascites and a pleural effusion were studied by tracing the abdominal fluid with I^{131}-albumin or Evans blue dye, and in 3, the alternate tracer was injected simultaneously into the pleural space. Total protein and albumin concentration of the serum and both effusions were determined initially, and the radioactivity and Evans blue concentration were determined in each fluid at intervals of from 2 to 50 hours after injection.

A direct shunt from abdominal to pleural cavities was indicated in 3 patients by pleural fluid concentrations of the abdominally injected tracer several times higher than the serum levels. In the only 2 patients with dyspnea, the shunt was functionally large, as indicated by rapid equilibration of the abdominal tracer in the pleural cavity. In 2 patients, the concentration of the tracer in the pleural fluid was consistently below that of the plasma, indicating diffusion by way of the blood rather than by the diaphragm. This was the case in the 3 instances in which the alternate tracer was injected into the pleural space; there was no evidence of a thoracoabdominal flow of fluid. After 48 hours, concentrations of isotope and dye were equal when corrected for albumin in all 3 spaces.

In conclusion, pleural effusion occurs commonly in severe cirrhosis and not infrequently results from upward passage of fluid through the diaphragm.

Percutaneous Splenic Pulp Manometry as an Aid in the Diagnosis of Acute Upper Gastrointestinal Bleeding

By *William F. Panke, Augusto H. Moreno, Louis M. Rousselot and William J. Grace.* Departments of Surgery and Medicine, St. Vincent's Hospital, New York City.

Early diagnosis in patients actively bleeding from the upper gastrointestinal tract, especially when the bleeding is acute and severe, continues to present many problems to the physician. Of the major sources of upper gastrointestinal hemorrhage, esophagogastric varices are probably the most difficult to treat and are associated with the highest mortality. Since the management of these patients differs considerably from that of those patients bleeding from other lesions, knowledge of the existence or absence of varices is one of the most vital aims of emergency diagnostic methods.

Combined percutaneous splenic pulp manometry and splenic portography in a large series of patients has revealed satisfactory correlation between splenic pulp pressure and the extent of portal congestion and collateralization. This correlation suggested that splenic pulp manometry alone might have diagnostic value in cases of upper gastrointestinal bleeding. Clinical experience has shown that if splenic pulp pressure is elevated (greater than 350 mm. H_2O), a tentative diagnosis of bleeding esophageal varices is usually confirmed. If, however, the pressure is within normal (up to 250 mm. H_2O), it can be assumed that portal hypertension with varices does not exist. Patients with splenic pulp pressures between 250 and 350 mm. H_2O may or may not have portal hypertension. In this area, an overlap exists between normals and those with portal hypertension. In these patients, definitive diagnosis is dependent upon portographic findings, i.e., the presence or absence of hepatofugal flow. The great majority of patients who bleed from varices, however, have pressures in excess of 350 mm. H_2O, and not uncommonly in the range of 400 to 600 mm.

It is felt that the procedure of splenic pulp manometry has several advantages over other diagnostic methods such as barium x-ray studies, liver chemistries, trial balloon tamponade and endoscopy.

IMMUNOLOGY

The Effect of 6-Mercaptopurine on Immune Responses

By *Robert Schwartz, Anna Eisner and William Dameshek*. Blood Research Laboratory, New England Center Hospital, Boston, and Department of Medicine, Tufts University School of Medicine.

The formation of antibodies involves the synthesis, in relatively large amounts, of a new type of protein. It is possible that the process of antibody formation is associated with hypermetabolism of the tissues producing such protein. If such is the case, then these rapidly metabolizing tissues might be susceptible to the action of antimetabolites. Reasoning along these lines, we decided to determine if 6-mercaptopurine, a powerful nucleic acid antagonist, would affect antibody production in rabbits.

Pure primary and secondary responses were provoked in rabbits by the intravenous injection of I^{131}-labelled human serum albumin. The rate of disappearance of radioactivity from the plasma, the appearance of circulating antigen-antibody complexes and the amount of humoral antibody were determined. Radioactivity disappeared from the plasma of the control, primary response animals in 3 phases: (1) an initial drop within 24 hours, representing equilibration between intra- and extravascular compartments; (2) a logarithmic decay lasting about 6 days and representing normal protein catabolism; (3) a sudden "immune disappearance" beginning around day 7, accompanied by the appearance of circulating antigen-antibody complexes and followed shortly by the presence of humoral antibody. 6-Mercaptopurine–treated animals did not develop the "immune disappearance" phase; the injected protein disappeared logarithmically over an 18-day period. Neither antigen-antibody complexes nor humoral antibody were detected in this group. Both treated and control animals developed classical anamnestic responses. That the effect on the primary response was not due to interference with phagocytic activity was determined by comparing the rates of disappearance of Cr^{51}-labelled heterologous red blood cells from the blood of normal, 6-mercaptopurine–treated and thorium-blocked rabbits. The labelled red blood cells disappeared almost entirely within 24 hours in the first 2 groups, while they were detectable in the blood of the thorium-blocked animals after 8 days.

6-Mercaptopurine will thus completely abolish the primary immune response, but it has no effect on the anamnestic reaction. The inhibition of the primary response is not due to an alteration of phagocytosis.

Observations on Auto-Immunity in Thyroid Disease

By *Martin J. Cline, Marcus S. Brooke and Herbert A. Selenkow*. Department of Medicine and Department of Bacteriology and Immunology, Harvard Medical School, and Department of Medicine, Peter Bent Brigham Hospital, Boston.

The traditional concept that the body does not form antibodies against its own tissues has recently been challenged. Studies on lymphoid thyroiditis and other thyroid disorders in animals and in man now permit a more factual basis for the concept that auto-immune processes may cause systemic disease. This investigation was undertaken to determine the presence and persistence of thyroid antibodies in chronic lymphoid thyroiditis and certain other thyroid disorders.

The tannic acid hemagglutination technic was used to assay sera of patients for the presence of antibodies against extracts of thyroid, liver, lung and kidney tissues and against purified thyroglobulin. Results of these studies showed significant antibody titers in 14 of 16 patients with chronic lymphoid thyroiditis (Hashimoto's struma) to extracts of thyroid gland or to purified thyroglobulin, but none to kidney, liver or lung extract. Utilizing zone electrophoresis on cellulose columns, the serum antibodies from 2 patients with lymphoid thyroiditis were localized in the gamma globulin fraction. One of these patients who had markedly elevated serum levels of gamma globulin and an extremely high antibody titer was found to have a biologically false serologic test for syphylis. Two patients with acute thyroiditis and one with subacute thyroiditis exhibited elevated antithyroglobulin titers. Of 20 patients with miscellaneous thyroid disorders, 4 had demonstrable antibodies to thyroglobulin; 3 of these had received radioactive iodine therapy for hyperthyroidism.

The hypothesis is proposed that various nonspecific processes which may liberate thyroglobulin into the circulation are responsible for the initiation of an auto-immune process which may contribute to the pathogenesis of lymphoid thyroiditis and other thyroid disorders.

Experience with Labeled Antiglobulin Technics in Detection of Lupus Factor

By *George J. Friou.* Medical Service, V. A. Hospital, West Haven, Connecticut, and Department of Internal Medicine, Yale University Medical School.

Two technics reported earlier utilize labeled anti-human globulin to detect the reaction of lupus globulin factor with nucleoprotein. In one, fluorescent antiglobulin is used to detect lupus globulin bound to nucleoprotein spots on glass slides. Calf thymus nucleoprotein, prepared under suitable conditions, is more stable than indicated by earlier experience. It has also been found that artificial complexes of commercial DNA and histone may be used. Preparation of fluorescent conjugates has been greatly simplified (Marshall et al., Proc. Soc. Exp. Biol. & Med. 98:898, 1958). Results may be read with the naked eye using generally available ultraviolet lamps (Wood's lamp). This method has therefore become more generally applicable and is especially useful as a qualitative test.

The other procedure is similar in principle but uses iodine^{131} as the label on the antiglobulin. Some conditions influencing reaction are: size of coated area, lupus factor concentration, temperature and pH. Antibody concentrations have been held constant, and results indicate presence of antibody in excess. For clinical tests, 1 cm. glass cylinders coated inside and out are used. Serum is diluted to 10% or 2% in pH 6.5 phosphate buffer. Incubation is in 2 ml. volume overnight at 0 C., using solid glass insert to raise serum level. Incubation with antibody is 4 hours. Results are expressed as units, relative to a standard serum. In proper range, counts per minute relate well to relative lupus globulin concentration.

Correlation of lupus factor content of serums with clinical findings of patients when studied over a period of time indicates a general relationship between level and continued disease activity. Results suggest that lupus globulin level measurement may be useful in long-term management of DLE.

Antibodies against Nucleoprotein Extracts in Patients and Animals

By *Howard C. Goodman and Robert Bowser.* National Heart Institute, Bethesda, Maryland.

The demonstration by use of the fluorescein-labeling technic that gamma globulins from the sera of patients with systemic lupus erythematosus localized on nuclei of white cells and other tissues suggested the use of serologic methods to detect this reaction. The method of Chargaff and Davidson (*The Nucleic Acids,* Academic Press, N. Y., 1955) for low ionic strength extraction of nucleoproteins was applied to human liver, rabbit liver and calf thymus. The distilled water extracts were diluted in saline buffered at pH 7.2, and the titer of "antibodies" to the extracts were determined by use of the Boyden tannic acid hemagglutination technic. Titers against human and rabbit nucleoprotein extracts of from 1:40 to 1:10,000 were found in 12 of 22 sera from patients with S.L.E. In 19 of the 22 sera titers of 1:80 to 1:100,000 were found against calf thymus nucleoprotein extracts. Sixty sera from patients with a variety of other diseases were negative.

Immunization of rabbits with rabbit liver nucleoprotein extracts did not produce serologically detectable antibodies against the nucleoprotein extracts. Seven rabbits were then immunized with human nucleoprotein extracts in Freund's adjuvant. All 7 rabbits developed antibodies not only to *human* liver nucleoprotein extracts but also to *rabbit* liver nucleoprotein extracts. These antibodies were detectable by precipitation and complement fixation technics as well as by the tanned cell hemagglutination test. These results are in accord with the hypothesis that antibodies made against foreign nuclear material (bacterial) could produce the antibodies against native nuclear material which are detectable in patients with systemic lupus erythematosus.

INFECTIOUS DISEASE

A Study of Antibiotic Prophylaxis in Patients with Acute Heart Failure

By *Robert G. Petersdorf and Richard K. Merchant.* University Medical Service, Yale–New Haven Medical Center, and Department of Medicine, Yale Medical School.

Patients with acute heart failure appear to be particularly susceptible to the development of pneumonia, which frequently is difficult to differentiate from failure by physical examination or x-ray. This study was performed to ascertain whether prophylactic administration of antibiotics would prevent pulmonary infections in these patients.

Using a double-blind technic, 72 randomly selected patients were given 2 Gm. of chloramphenicol daily for a week; 78 received placebo. The groups were comparable with respect to age, sex, race and etiology for congestive failure. There was no difference in the clinical course as measured by venous pressure, circulation time, vital capacity, weight loss and symptomatic improvement. Fever attributable to heart failure was present in 42% and occurred more frequently in the controls. Leukocytosis on the basis of failure was unusual.

Thirty-eight patients died, 21 in the antibiotic and 17 in the placebo group; 8 in the former and 6 in the latter had pneumonia clinically or at autopsy. Of these 14, 4 recovered after administration of penicillin (3 placebo, 1 chloramphenicol) and 10 expired (3 placebo, 7 chloramphenicol). In 6 patients, pulmonary infection was incidental to other causes of death, but in 4 (1 placebo, 3 antibiotic) it was a major factor.

Pneumonia was present in 10 of 28 patients in whom it was suspected roentgenologically and was missed by the radiologist only twice in 112 patients. Other clues indicative of pneumonia plus heart failure are purulent sputum, fever above 101 F. for at least 3 days and leukocyte count over 15,000.

Adverse reactions to chloramphenicol occurred in 6 patients, 2 of whom developed severe staphylococcal enterocolitis. These observations do not support the suggestion that antibiotics be given routinely to patients with congestive failure. Instead, special care should be taken to discover pulmonary infections early and, once detected, to institute appropriate antimicrobial therapy.

Lysozyme Release as an Indication of in Vivo Leukocyte Injury by Endotoxin

By *John C. Ribble and Ivan L. Bennett, Jr.* Departments of Medicine and Pathology, Johns Hopkins University School of Medicine, Baltimore, Maryland.

Two known effects of endotoxin—the production of the Shwartzman reaction and the release of circulating endogenous pyrogen—do not occur in animals made agranulocytic with nitrogen mustard. It has been postulated that damage to granulocytes during leukopenia produced by endotoxin releases substances essential to these reactions. Leukocyte damage by endotoxin has not been shown in vivo, but Kerby has demonstrated injury in vitro by showing the leakage of lysozyme from granulocytes incubated with endotoxin. The purpose of this study was to estimate white cell damage in vivo by demonstrating release of lysozyme into the plasma after injection of endotoxin.

Samples of plasma were obtained before and at varying intervals after injection of 0.2 ml. of typhoid vaccine. Lysozyme concentrations were measured by determining photometrically the rate of clearing of mixtures containing plasma and turbid suspensions of *Micrococcus lysodeikticus.*

In normal rabbits, there was no detectible change in lysozyme activity 5 minutes after the injection of typhoid vaccine, but at 120 minutes the mean activity had increased to a maximum of 240% of the preinjection level, and by 240 minutes the activity was still 200% of the initial value. Similar increases were noticed after injection of highly purified endotoxin. No change occurred in control animals injected with saline alone.

Only a slight increase (135% of initial value) was noted at any time after injection of typhoid vaccine into animals made agranulocytic with nitrogen mustard, evidence that leukocytes were a major source of the lysozyme released into the plasma. Similarly lysozyme activity failed to rise in animals injected daily for 2 weeks prior to challenge with increasing doses of typhoid vaccine (tolerant rabbits).

These results support the hypothesis that white cells from normal animals are damaged in vivo by endotoxin, but leukocytes from tolerant animals are protected from this injury.

KIDNEY

Renograms Using I^{131}-labelled Diodrast—a Test for Unilateral Renal Disease

By *Edward D. Frohlich, Frank J. Fedor and Edward D. Freis.* Mt. Alto V. A. Hospital, and Department of Medicine, Georgetown University School of Medicine, Washington, D. C.

There is a need for a single, atraumatic screening test for estimating unilateral renal function, the only such method available at present being the intravenous pyelogram. The purpose of this study is to evaluate the technic of Taplin and Winters by counting over the renal areas following the intravenous injection of I^{131}-tagged Diodrast.

Eighty-six "renograms" were recorded in 75 patients at this hospital. Of these, 27 were normal controls. None of these showed abnormal renograms. Twenty-six patients had suspected or known renal disease without hypertension, of which 20 exhibited unilateral abnormal renograms. Of that number, 18 were proven to have unilateral renal disease by other tests or surgery. The remaining 2 had bilateral disease, but no distinguishing tests were done to differentiate degrees of involvement of each kidney.

Twenty-seven roenograms were obtained in 22 patients with hypertension. Ten had normal renograms and normal renal function tests. Nine showed bilaterally abnormal records corroborated by other renal function studies. Three patients showed abnormal unilateral renograms. Howard tests were negative in 2. These patients are still being studied and will have aortograms in an attempt to confirm or reject the renographic diagnosis of unilateral renal disease. All 3 showed abnormal patterns on the right where results may be obscured by hepatic uptake of the Diodrast. This difficulty has been largely circumvented by (1) wide-field collimation; (2) right angle rather than caudal direction of the probe; (3) addition of nonradioactive carrier Diodrast to aid in saturating plasma albumin binding sites; and (4) most important, preliminary exploration of the renal area to find the site of maximum clearance using a small test dose of I^{131}-Diodrast.

It appears that this method may be useful in detecting unilateral renal disease. In no case was there an abnormal intravenous pyelogram and normal renogram.

The Effect of a Low-Potassium Diet on the Renal Response to Respiratory Acidosis

By *Howard Levitin, David Beck and Franklin H. Epstein.* Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut.

When rats are exposed to 8% carbon dioxide for 24 hours, urinary excretion of chloride increases, in association with an increase in the excretion of ammonium and potassium. If chloruresis induced by respiratory acidosis were secondary to alterations in the tubular transport of cations or the excretion of ammonium, one might expect that it would be modified in potassium-deficient rats, since in such animals the pH of renal tubular cells in decreased and ammonium excretion is enhanced. This possibility was explored by studying rats that had been maintained on a low-potassium diet for 3 weeks. At the end of this time, the animals were slightly alkalotic, their serum potassiums were depressed, they excreted less than 5 mEq./L. of potassium in the urine, and their mean daily urinary excretion of ammonium exceeded that of control rats on a regular diet by 70%.

Exposure to 8% CO_2 for 24 hours induced a negative balance of chloride similar in magnitude in both potassium-deficient and control rats. The urinary excretion of ammonium was increased in both groups by exposure to CO_2. No change in the balance of potassium was noted in the potassium-deficient group, but these animals developed a slight negative balance of sodium when placed in CO_2. In contrast, rats on a normal diet lost potassium, but not sodium, in conjunction with chloruresis.

These data are compatible with the hypothesis that the increased renal excretion of chloride induced by respiratory acidosis is a direct result of altered tubular handling of chloride by the renal tubules rather than a secondary consequence of changes in the excretion of ammonium or the renal transport of cations.

The Effect of Strophanthidine on Electrolyte Metabolism and PAH Accumulation in Rabbit Kidney Slices

By *Maurice Burg and Jack Orloff.* Laboratory of

Kidney and Electrolyte Metabolism, National Heart Institute, Bethesda, Maryland.

The effect of the cardiac aglycone strophanthidine on PAH accumulation and electrolyte metabolism in thin slices of rabbit renal cortex has been investigated using the incubation technic described by Mudge. In medium containing 5 mM/L. potassium, 3 x 10^{-5} M strophanthidine decreased mean slice potassium concentration from 69.2 mEq./Kg. wet weight to 59.8 and increased mean slice sodium from 78.1 to 89.8 mEq./Kg. The fall in potassium concentration was due to a decrease in potassium influx without change in potassium efflux. PAH slice to medium ratio at 1 hour was reduced from an average of 10.1 to 5.6. Oxygen consumption, which was initially 1.24 μL./100 mg. wet weight/hr., decreased 0.19 in the controls and .21 in the slices exposed to strophanthidine. The effectiveness of the drug was a function of medium potassium concentration. Strophanthidine-induced changes in PAH uptake and slice electrolyte were significantly greater at 2 mM/L. of medium potassium than at 60 mM/L.

Decreased PAH uptake following strophanthidine may be secondary to potassium loss from the slice since low slice potassium concentration alone is sufficient to diminish PAH accumulation. Alteration of tissue electrolyte composition by cardiac glycosides without significant change in oxygen consumption has been observed in other tissues. In red cells, similar changes in electrolyte metabolism are principally due to inhibition of linked sodium-potassium exchange, with resulting decrease in potassium influx and sodium efflux. Decreased effectiveness of the cardiac glycosides at high medium potassium levels has also been noted in the red cell and attributed to competition between the drug and potassium.

Since the effects observed are analogous to those in the red cell and other tissues, it is probable that strophanthidine exerts its renal effect by a similar mechanism, namely, by a direct interference with a linked sodium-potassium exchange process.

Glomerular Perfusion during Acute Renal Insufficiency from Mercury Poisoning in the Rat

By *Ethan A. H. Sims, Herbert I. Goldberg, Joseph R. Kelley and Burton A. Sisco.* Department of Medicine, College of Medicine, University of Vermont.

Following the initial insult in acute renal insufficiency (acute tubular necrosis), there ensues a period of oliguria or anuria. There is conflicting evidence as to whether glomerular perfusion continues to be sharply reduced during this period and as to whether a return of, or increase in, glomerular perfusion is coincident with the onset of diuresis.

To clarify this point, study has been made of the number of glomeruli actively perfused at 1-, 2-, 3-, 4- and 7-day intervals after induction of acute renal insufficiency by administering subcutaneously 10 mg./Kg. of mercuric chloride to the rat. Thioflavin-S, a flourescent dye adhering to the intimal surfaces of blood vessels, was injected intravenously, and 30 seconds later a kidney was removed, sectioned in a median longitudinal plane, fixed in glycerin and examined microscopically under incident ultraviolet light. Urine volume was measured and microscopic sections of the kidneys were made.

In 18 control rats, the average number of glomeruli visualized per $mm.^2$ of sectioned renal cortex was 18.5 ± S. D. 1.05, with a range of 15 to 21. In the group of 36 surviving rats given mercuric chloride, counts of perfused glomeruli were reduced to an average of 11.2/$mm.^2$ with a range of 8 to 18. In the separate groups there was no correlation between the number of days following administration of mercury and the glomerular count. Neither was there correlation between the daily urine volume, which varied from 0 to 60 ml./day, and the glomerular counts. There was likewise a lack of correlation between the degree of tubular damage noted in the histologic sections of the kidneys poisoned with mercury and the corresponding counts of active glomeruli.

It is concluded that in experimental poisoning with mercury in the rat, the maintenance of anuria is not dependent upon a critical reduction in glomerular persusion and that diuresis may take place without a change in this perfusion. It is suggested that the oliguria is a reflection mainly of renal tubular damage and that the oliguria is explained by increase in back-diffusion rather than by decrease in glomerular filtration secondary to glomerular ischemia.

A Possible Mechanism for Renal Hypertension in Man

By *John P. Merrill, Alberto Guinand-Baldo and Carmelo Giordano.* Department of Medicine, Harvard Medical School, and Peter Bent Brigham Hospital, Boston.

In the past, much evidence for the kidney's role in the production of hypertension has been obtained from animal experiments in which impairment of blood supply or the functioning mass of one kidney occurred in the presence of a normal contralateral kidney with subsequent removal of the damaged organ (Group I). Other studies have been concerned with the production of "renoprival" hypertension or vascular disease in bilaterally nephrectomized animals (Group II). These two groups of experimental situations have been studied in the human under fortuitous clinical conditions. *Group I:* Three patients with hypertension and renal disease who successfully received a normal kidney from a healthy identical twin. *Group II:* Three patients without renal tissue maintained for 51 days or more following the accidental removal of a congenital single kidney.

Following transplantation of a single normal functioning kidney, blood pressure dropped to near normal levels, but vasomotor lability and hyperreactivity to cold pressor stimuli, Valsalva overshoot and standard norepinephrine infusion remained. Following the removal of the diseased kidneys, the "hyperreactivity" disappeared. As control, a patient receiving a nonrelated kidney homograft functioning subnormally for 5½ months, showed no drop in blood pressure. The level of blood pressure and the degree of sensitivity varied with the sodium intake. In the hypertensive phase, renin concentrations were not elevated, nor was there marked difference in the ability to excrete infused norepinephrine. *Group II:* None of the "renoprival group" showed elevation of blood pressure or hypersensitivity to the pressor stimuli except as clearly related to excess sodium and water intake. A possible interpretation of these results is that the damaged kidney first sensitizes to normally occurring pressor stimuli. This degree of sensitivity depends to some extent upon the sodium intake. With further reduction of functioning renal parenchyma, clinical hypertension and vascular disease appear. In the normally hydrated human, it appears that a damaged kidney rather than absence of renal tissue alone is the prerequisite to the development of "renal hypertension."

Mechanisms of Hyperlipemia in Experimental Nephrosis

By *Norman Kalant and Judith Saffran.* Research Laboratory, Jewish General Hospital, Montreal, and Department of Investigative Medicine, McGill University.

There are 3 current hypotheses to explain the development of hyperlipemia in experimental nephrosis: increased hepatic synthesis of lipid, increased mobilization from depots and decreased removal from plasma by liver. This problem has been reinvestigated using radioactive tracer technics. Normal rats were given injections of acetate-1-C^{14} to permit endogenous labelling of body fats. Some of the animals were then injected with anti-kidney serum to produce nephrosis and the distribution of radioactivity among body lipid compartments measured at intervals during the development of hyperlipemia. In normal rats, the specific activity of serum fatty acids rapidly approached that of depot acids, while in nephrotic animals it fell at the same rate as liver acids. This has been interpreted to indicate that the excess lipid in the serum is derived from the liver.

After intravenous injection of palmitic acid-1-C^{14} complexed with albumin, rats with established nephrosis disposed of the radioactivity from the serum at a normal rate. The radioactivity later reappeared in the circulation, probably as esterified fatty acid, to a greater degree in nephrotic than in normal animals. Serum nonesterified fatty acid concentrations were not significantly different in the two groups.

These experiments indicate an increased synthesis and release of fatty acids by the liver.

After intravenous injection of C^{14}-labelled chylomicrons, radioactivity disappeared from the plasma of nephrotic rats at a much lower rate than normal, suggesting a defect in lipoprotein lipase activity in experimental nephrosis.

The hyperlipemia of experimental nephrosis appears to be due to at least two abnormalities: prolonged elementary lipemia and increased hepatic synthesis and turnover of lipids.

The Effect of Antidiuretic Hormone on the Permeability of the Toad Bladder

By *Roy H. Maffly, Richard M. Hays, Ezra Lamdin and Alexander Leaf.* Departments of Medicine, Massachusetts General Hospital, and Harvard Medical School, Boston.

The urinary bladder of the toad actively transports sodium from its mucosal to its serosal surface. Mammalian antidiuretic hormone stimulates this transport by 200–300%. Since Ussing has given evidence that the action of the antidiuretic hormone on the frog skin is to open pores, we have attempted to characterize the effect of the hormone on the permeability of the toad bladder to substances other than sodium ion.

With the toad bladder mounted as a diaphragm separating two halves of a chamber, and short circuited so that no electrochemical gradients existed across the membrane, a radioactively labelled substance was placed on one side and its rate of appearance on the other side of the membrane measured. From this, a permeability coefficient for the substance was calculated. The effect of the addition of pitressin on this permeability was determined.

Although the toad bladder is permeable to some extent to inorganic ions (K^+, Cl^-, $SO_4^=$) and also to some organic compounds (lactate, glycine, sucrose, inulin), with permeability coefficients in the order of .5–5 x 10^{-7} cm./sec., the addition of pitressin has no effect on this permeability in either direction across the membrane. Urea, however, has a greater permeability coefficient (25 x 10^{-7} cm./sec.) that is markedly increased (to 300 x 10^{-7} cm./sec.) by pitressin. Thiourea, which is structurally very similar to urea, nonetheless is not affected by pitressin. Methanol and water have very large permeability coefficients (*circa* 500 x 10^{-7} cm./sec.) but are affected little or not at all by pitressin.

These results are consistent with the concept that the action of antidiuretic hormone is to enlarge channels to which only a limited number of compounds have access. It appears possible that the active transport of sodium, bound in an as yet unidentified carrier-complex, is performed through these same channels.

The Relationship of Urinary pH to the Management of Chronic Infections of the Urinary Tract with Organic Acids and Bases

By *D. Zangwill, A. Kaitz, R. Cotran, P. Porter and E. Kass.* Mallory Institute of Pathology, Thorndike Memorial Laboratory, Second and Fourth (Harvard) Medical Services, Boston City Hospital, and Departments of Bacteriology and Immunology, and Medicine, Harvard Medical School, Boston.

The treatment of chronic infections of the urinary tract has been generally unrewarding. An investigation of the activity of certain organic acids and bases as antibacterial agents has been undertaken with the hope that by proper control of pH these substances would provide effective bacteriostasis in the urinary tract.

Patients with chronic infections of the urinary tract were studied by using bacterial counts of the urine. As previously reported, the ingestion of 5–15 Gm. of methionine daily lowers the urinary pH to 4.5–5.0. With proper acidification, agents such as methenamine mandelate or hippuric acid lowered the bacterial counts significantly in ⅔ of patients. The effectiveness of treatment could be shown to depend upon the pKa of the drug and its presence in the urine in sufficient concentration to supply the necessary number of unionized molecules. In 3 patients with proteus infections and alkaline urines, the nitrofuran 5-nitro, 2-furaldehyde,2 (2-dimethylaminoethyl) semicarbazone, with a pKb of 7.6, eliminated the proteus and decreased the bacteriuria.

With appropriate control of urinary pH, and sufficient quantities of active antibacterial drug, urine can be kept free of bacteria. The effect is occasionally curative but is more often only suppressive. Further study is necessary to determine whether this type of urinary bacteriostasis is beneficial to the renal phase of the infection.

NERVE AND MUSCLE

The Effect of Sodium Loading and Depletion on Muscular Strength and Aldosterone Excretion in Familial Periodic Paralysis

By *Peter T. Rowley and Bernard Kliman.* National Institute of Neurological Diseases and Blindness, and National Institutes of Arthritis and Metabolic Diseases, Bethesda, Maryland.

1. Two patients with familial periodic paralysis were investigated with regard to the effect of sodium loading and depletion on muscular strength and on resistance to measures disposing

to muscular weakness. Sodium loading was achieved by dietary excess or by administration of 2-methyl, 9-α-fluorohydrocortisone. Sodium depletion was accomplished by dietary restriction or by administration of a mercurial diuretic or SC-8109, a steroid-17-lactone with an action antagonistic to aldosterone.

2. Sodium loading was in general associated with a decrease, and sodium depletion associated with an increase, in strength and in resistance to measures disposing to weakness.

3. Urinary aldosterone excretion, measured by a double isotope derivative technic, was normal prior to two spontaneous attacks and, in a normal fashion, rose with sodium depletion and fell with sodium loading.

NEOPLASTIC DISEASE

The Value of 5-Fluorouracil in Human Cancer

By *Robert D. Sullivan, Edward Miller, Veronica B. Murphy and Richard Mechanic.* New York V. A. Hospital, and Sloan–Kettering Institute, New York City.

5-Fluorouracil, one of a new series of fluorinated pyrimidines synthesized by Heidelberger et al. (Nature *179*:663–666, 1957), has been demonstrated to have anti-tumor activity against a number of transplanted tumors in the mouse and rat. A preliminary study by Curreri et al. reported some comparable clinical activity in several forms of advanced cancer. Because of the profound toxic effects of this compound on the gastrointestinal tract, a study of its anti-tumor activity in patients with predominantly gastrointestinal malignancy was undertaken.

Thirty-six patients with advanced, incurable cancer received one or more courses of 5-fluorouracil. The average maximum tolerated intravenous course of therapy was 15 mg./Kg. daily for 8 days. The comparable oral doasge was 15 mg./Kg. daily for 10–14 days. Toxic manifestations usually developed toward the end of a course of therapy and consisted of: anorexia, nausea, vomiting, diarrhea, oral ulcerations, alopecia and hematologic depression. The drug was immediately discontinued when definite toxicity was apparent, and symptoms usually abated within 1–3 weeks.

Twenty-six patients were evaluated for anti-tumor activity. Twelve patients showed some *objective* evidence of tumor regression as follows: adenocarcinoma of the colon and rectum (6 patients), adenocarcinoma of the stomach (3 patients), nasopharyngeal carcinoma (1 patient), and metastatic carcinoma of unknown origin (2 patients). Fourteen patients failed to respond despite repeated courses of treatment. All patients who responded manifested moderately severe toxic reactions to the drug. Response was of short duration (2–10 weeks) and repeated courses of treatment were required to maintain improvement. In all cases, resistance eventually developed and further administration of the compound resulted in toxicity without benefit.

It is concluded that 5-fluorouracil has shown evidence of anti-tumor activity in several types of human cancer, but because of the severe toxic effects at therapeutic dosage levels, its practical application is restricted to carefully selected cases.

The Anti-Tumor Activity of Uracil Mustard, a New Alkylating Agent

By *Montague Lane and Margaret G. Kelly.* Clinical Pharmacology and Experimental Therapeutics Section, National Cancer Institute, Bethesda, Maryland.

Since the initial report of the actions of nitrogen mustard, numerous derivatives of this alkylating agent have been synthesized and evaluated as potential anti-tumor agents. Recently, Lyttle and Petering synthesized 5-bis-(2′-chloroethyl)-aminouracil (uracil mustard) and indicated that it possessed anti-tumor activity. We have made quantitative comparisons of the therapeutic effects of uracil mustard and HN2 against advanced tumors.

Uracil mustard was stable in 10% ethanol–90% physiologic saline for at least 4 hours. The acute LD_{50} (i.p.) was approximately 6 mg./Kg. for mice and 2 mg./Kg. for rats. The oral LD_{50} was 1.2–2 times higher. Therapeutic doses in mice caused no weight loss but produced leukopenia in 3–5 days with recovery by day 7. The acute oral LD_{50} was 0.9 mg./Kg. in dogs. Supralethal doses caused apathy, anorexia, weight loss and leukopenia within 3–7 days and death within

4–15 days. Marrow depression and hemorrhages were found at autopsy. Dogs tolerated 0.65 mg./Kg. once a week for 3 weeks.

Animals bearing a spectrum of tumors were treated with uracil mustard (p.o. or i.p.) or HN2 (i.p.). Treatment was begun shortly before the first deaths in control groups. HN2 was considerably less effective in prolonging survival. The increases in median survival obtained with uracil mustard were: leukemia L-1210, 50%; Dunning rat leukemia, 183%; Dunning rat lymphosarcoma, 137%, with 50% complete regression; mouse reticulum cell sarcoma 6867, 80%; lymphoma K4, 200%; lymphosarcoma L 2, 280%, with 70% complete regression. Also, uracil mustard decreased the growth of advanced Walker carcinoma 256 (70%) and mammary carcinoma 241–6 (82%).

To summarize, uracil mustard produced a marked therapeutic effect against a variety of advanced animal tumors and in these situations appeared superior to HN2. In addition, these effects of uracil mustard were obtained upon oral as well as parenteral administration.

RESPIRATORY SYSTEM

Pulmonary Pressure–Volume Relationship in Thoracic Deformity (Kyphoscoliosis)

By *Er Yi Ting and Harold A. Lyons.* State University of New York, Downstate Medical Center, College of Medicine, Brooklyn.

The pressure-volume diagram of the total respiratory system by the method of Rahn et al. has been used for patients with various pulmonary diseases. The method of study gives information about the mechanics of breathing. It allows for the analysis of the separate components, the thorax and lung, which summate the total pressure-volume relationship.

Eight unselected patients with scoliosis or kyphoscoliosis were included in the study. Spirometrically all of the patients showed evidence of restrictive type of pulmonary disease. By measuring the intrapulmonary pressure exerted against a water manometer at different lung volumes when all muscular activity was voluntarily relaxed, a relaxation pressure curve (total pressure P_R) was obtained. In patients, the mean slope became horizontal, shifted downward and to the right. Measuring the intraesophageal pressure at points of no flow and at different intervals, the lung pressure curve (P_L) was recorded. By use of the equation $P_R = P_L + P_C$ and $P_C = P_R - P_L$, a thoracic pressure curve (P_C) was arithmetically determined. A plot of these 3 curves, using lung volumes against pressure, constructed a complete relaxation pressure volume diagram for each study. The compliance of the lung and thorax and the total compliance were determined from these volume pressure curves.

Mean values for the control group of 6 normal subjects were: Total compliance, 0.085 (±.009) L./cm. H_2O; lung compliance, 0.165 (±.021) L./cm. H_2O; thoracic compliance, 0.153 (±.029) L./cm. H_2O. The mean values for the 8 patients were: Total compliance, 0.032 (range .018–.061) L./cm. H_2O; lung compliance, 0.082 (range .039–.140) L./cm. H_2O; thoracic compliance, 0.057 (range .024–.110) L./cm. H_2O.

From the data, patients with deformity of the thoracic cage were found to have respiratory levels with units of intrapulmonary and intrapleural pressures which contrasts with the findings of the normal subjects. Lung compliance was found decreased, but the thoracic compliance was reduced to a greater degree, which reflected the marked fixation and non-expansibility of the thoracic cage. This alteration of the thoracic cage was the major factor responsible for the reduced total compliance.

An Objective Technic as an Aid in the Diagnosis of Acute Pulmonary Embolism

By *Eugene D. Robin, David M. Travis, Desmond G. Julian and Charles H. Crump.* Peter Bent Brigham Hospital, Boston.

The diagnosis of acute pulmonary embolism is difficult. Most diagnostic criteria are either non-objective or non-specific. This paper will describe a physiologic technic which may assist this difficult diagnosis.

In normal subjects, peak end-tidal expiratory CO_2 tension ($P_{A_{CO_2}}$) is essentially equal to arterial CO_2 tension ($P_{a_{CO_2}}$). Pulmonary embolism without infarction produces a portion of alveolar space which will be ventilated but no longer perfused

by pulmonary capillary blood. There follows a decrease in $PACO_2$ as compared with $PaCO_2$. It can be shown that:

$$\% \text{ of lung ventilated but unperfused} = \frac{PaCO_2 - PACO_2}{PaCO_2} \times 100.$$

Experimental pulmonary artery occlusion in dogs has demonstrated that close agreement exists between the predicted and calculated extent of occlusion.

In 24 normal subjects, the mean ($PaCO_2$ – $PACO_2$) difference was 0.1 ± 1.8 mm. Hg. Of 11 patients with suspected pulmonary embolism, 8 showed significant ($PaCO_2$ – $PACO_2$) differences. Four had conclusive evidence by x-ray, surgery or autopsy, and 4 had clinical evidence of the diagnosis. Three patients showed no significant ($PaCO_2$ – $PACO_2$) differences: one of these showed no occlusion of the pulmonary arteries at autopsy.

Caution must be exerted in the interpretation of results because of certain inherent limitations: (1) technical problems in measuring $PaCO_2$ and $PACO_2$; (2) lung ventilated but unperfused must exceed about 12% to be significant; (3) infarction will minimize the ($PaCO_2$ – $PACO_2$) difference; (4) ($PaCO_2$ – $PACO_2$) differences may exist due to abnormal ventilation—perfusion relationships occurring in pulmonary disease; (5) the bronchial circulation will develop collateral supply after 2 weeks.

Despite these limitations, this technic has proved useful in evaluating the possibility of pulmonary embolism. Like most tests in clinical medicine it must be used in association with other findings and its limits of error recognized.

THERAPEUTICS

A Controlled Study of the Anti-Inflammatory Effect of Buccal Streptokinase

By *Philip S. Norman and Paul J. Kadull.* Department of Medicine, Johns Hopkins Hospital, Baltimore, and U.S. Army Biological Warfare Laboratories, Fort Detrick, Frederick, Maryland.

Partially purified streptokinase administered buccally has been reported to have an anti-inflammatory effect in local infections, in thrombophlebitis and at operative sites. These reports are based on uncontrolled clinical studies and have not yet been confirmed. In those controlled experiments which seemed to demonstrate anti-inflammatory effects in animals and man, enzymes have always been given parenterally. An attempt has been made to confirm the anti-inflammatory action of buccal streptokinase in man by a controlled double-blind study. A standard inflammatory stimulus was given in the form of a subcutaneous injection of an alum-precipitated anthrax vaccine to a group of volunteers known to have a 20 to 30% rate of local allergic reactions to previous injections of the vaccine. Two-hundred men and women received 40,000 units of streptokinase (Varidase) in buccal tablets or indistinguishable placebos on the same day they received the anthrax vaccine. There were 33 local inflammatory reactions to the vaccine in the treated group and 31 reactions in the untreated group. In both groups the reactions lasted for about 2 days and were of similar intensity. The two worst local reactions occurred in treated persons. An additional 100 individuals were pretreated for 3 days with 30,000 units of streptokinase a day or given placebos. After injection of anthrax vaccine on the third day, 8 reactions occurred in treated persons and 9 in untreated. Again the intensity and duration of the reactions were similar in each group, and the two most severe reactions occurred in treated subjects. Thus, it was not possible to demonstrate an anti-inflammatory effect in man when buccal streptokinase was given in a strictly controlled experiment. There were no significant side effects of the drug.

RHEUMATIC STATES

Latex Fixation in Nonrheumatic Diseases

By *Ellis Dresner, Patricia Trombly and Gerald F. O'Brien.* Medical Service, Lemuel Shattuck Hospital, Boston, and Department of Medicine, Harvard Medical School.

The agglutinating factor for globulin-sensitized erythrocytes and latex particles present in rheumatoid sera has been thought specific for this and closely allied diseases. To test this specificity, serologic agglutinating activity was studied by highly sensitive technics in nonconnective tissue disorders and the incidence compared with that in rheumatoid arthritis (RA) and disseminated lupus erythematosis (DLE).

Agglutinating activity was determined by latex fixation (LF) tests on whole sera and also on their reconstituted dialysed euglobulin fractions. The euglobulin fractions were also tested for their capacity to inhibit agglutination by known positive sera. Marked enhancement of latex fixation was found in the euglobulins as compared with whole sera, but the absence of inhibitor in the euglobulins was found the most sensitive test of activity.

(1) LF tests were negative by all 3 methods in 40 healthy subjects. (2) In 83 patients with RA, LF tests were positive in whole sera in 67%, in the euglobulins in 94%, and by the inhibition procedure in 97%. (3) Of 12 patients with DLE, 50% were positive in sera and 83% in the euglobulins and by the inhibition procedure. (4) All but 2 of 73 positive sera from patients with nonrheumatic disorders fell into one of two groups: (a) Of 54 patients with liver disease 31% were positive in the sera, 83% in the euglobulins and by the inhibition procedure. Of 11 patients retested, 10 remained positive. (b) Of 17 patients with miscellaneous virus infections, 94% had positive tests in their euglobulins, but only 24% were positive in whole sera. Of 8 patients retested, 4 became negative.

The sera from each of the positive groups failed to inhibit agglutination by sera from the other groups.

In conclusion, the "rheumatoid" agglutinating factor cannot be considered specific for RA and related diseases. A serologically identical factor was found in most cases of liver disease and during viral infections.

Sydenham's Chorea: Relationship of its Recurrences, as Isolated Manifestations, to Preceding Streptococcal Infections

By *Angelo Taranta.* Irvington House, Irvington-on-Hudson, New York.

Sydenham's chorea may occur as an isolated disease or in association with rheumatic polyarthritis and/or carditis. In the latter form, etiology has generally been thought to be streptococcal, and recent data have demonstrated that the time interval between the streptococcal infection and the chorea is longer than the interval between the same infection and the onset of the arthritis and/or carditis. When the chorea has occurred as an isolated process, its etiology has been obscure. The present study was designed to test the hypothesis that isolated chorea, too, follows streptococcal infections by an interval longer than polyarthritis and carditis.

Sixty children who had had Sydenham's chorea, and were therefore susceptible to recurrences, have been followed over a 6-year period in 2 anti-streptococcal prophylaxis clinics. A unique feature of these clinics has been the routine periodic testing of throat cultures and streptococcal antibodies. This has permitted the detection and dating of even sub-clinical streptococcal infections with high reliability.

There were no recurrences of chorea in the 41 children who did not have streptococcal infections. Of 19 patients who had one or more streptococcal infections, 3 had recurrences of chorea. The recurrences were not preceded or accompanied by rheumatic polyarthritis or carditis, and their clinical onset was 2½ to 6 months after the first immunologic evidence of streptococcal infection. They were not accompanied by high ESR or positive CRP test, except in one patient who had a second recurrence of chorea shortly after the first recurrence and an intervening streptococcal infection.

These data provide the first available evidence to indicate that Sydenham's chorea can follow Group A streptococcal infections by an interval longer than rheumatic polyarthritis and carditis, even in the absence of the latter manifestations and, indeed, in the absence of "rheumatic activity."

Incidence and Significance of the Rebound Phenomenon in Acute Rheumatic Fever

By *Alvan R. Feinstein, Mario Spagnuolo and Fred A. Gill.* Irvington House,, Irvington-on-Hudson, New York, and Department of Medicine, New York University College of Medicine.

The present investigation was done to obtain quantitative data regarding the "rebound" phenomenon in rheumatic fever. The rebound is defined as the reappearance of clinical and/or laboratory manifestations of rheumatic activity, in the absence of an intervening streptococcal infection, after these features have originally subsided.

The study included 261 consecutive patients admitted to Irvington House during the past 2 years for acute or convalescent treatment of unequivocal episodes of rheumatic fever. Anti-inflammatory therapy was given to patients in the following numbers: none, 33; salicylates, 105; steroids, 64; salicylates and steroids, 59. No rebounds occurred in 20, 51, 21 and 25 patients of each treatment group, respectively. A laboratory rebound (elevation in ESR or CRP values) occurred in 13, 43, 23 and 17 patients, respectively. A clinical rebound (fever, arthralgias or new cardiac signs together with laboratory abnormalities) occurred in 0, 11, 20 and 17 patients, respectively.

Laboratory rebounds appeared even in untreated patients and occurred usually within 8 weeks after therapy was stopped in the treated group. They all subsided spontaneously and had no evident clinical effects. Forty of the 48 clinical rebounds occurred during tapering or within 2 weeks after the cessation of therapy. They were more frequent in patients with valvulitis, particularly in those with definite cardiomegaly. They did not occur in untreated patients; in the treated groups, they were more prevalent in patients who received steroids. They seemed more frequent in patients treated with little or no penicillin. Although 8 of the 48 patients showed new cardiac signs with their clinical rebound, the change was permanent in only one.

The data indicate that a rebound occurs commonly after acute rheumatic fever and is generally a benign process. In untreated patients without rebounds, the maximum duration of ESR and CRP abnormalities was an average of 2 months. Long courses of anti-inflammatory therapy seemed to prolong the duration of rheumatic activity in many patients without necessarily improving the clinical course. It is therefore suggested that the initial course of treatment be less than 2 months.

PROGRAM, WESTERN SECTION

American Federation for Clinical Research

Wednesday and Thursday, January 28 and 29, 1959
Golden Bough Theater, Carmel, California

Dr. Richard J. Havel, Presiding

Presentations will be limited to ten minutes

WEDNESDAY, JANUARY 28

1:30 P.M.

BUSINESS MEETING

1:45 P.M.

1. **The Influence of Exogenous Iron on Hemoglobin Formation in the Premature Infant.**
   *Denman Hammond and Arlene Murphy,** San Francisco. *page 53*
2. **The Temperature of Venous Blood in the Extremities and its Relationship to the Clotting Process.**
   *Edward Rubenstein and Arthur Lack,** San Francisco. *page 61*
3. **In Vivo Metabolism of Tritiated Thymidine in Man.**
   *Joseph R. Rubini, Eugene P. Cronkite,*** *Victor P. Bond** *and T. M. Fliedner,** Upton, New York. *page 68*
4. **The in Vitro Incorporation of Nucleic Acid Precursors by Normal and Leukemic Human Leukocytes.**
   *J. L. Scott, G. E. Cartwright** *and M. M. Wintrobe,** Salt Lake City. *page 56*
5. **Quantitation of Gamma Globulin in Human Serum by Immunoprecipitation.**
   *Beach Barrett,** *Wade Volwiler*** *and Patricia Ann Wood,** Seattle. *page 58*
6. **Bone Marrow Transplant in Agammaglobulinemia.**
   Casimir A. Domz, Santa Barbara, California. *page 58*
7. **Gastric Ulceration in Experimentally-induced Polycythemia.**
   *James R. Dahl,** *Richard K. Blaisdell and Ernest Beutler,* Chicago. *page 70*
8. **Experimental Production of Atrophic Gastritis.**
   *Edward H. Storer and Francis C. Nance,** Memphis, Tennessee. *page 70*
9. **Tellurite Reduction as an Indicator of Staphylococcal Virulence.**
   *Paul D. Hoeprich, Garth F. Croft** *and Lionel M. West, II,** Salt Lake City. *page 73*
10. **A Medical and Psychosocial Study of Tuberculous Patients Who Relapse to Infectious Sputum during Hospitalization.**
    *James E. Hart,** *Joan K. Jackson** *and Thomas H. Holmes,* Seattle. *page 74*
11. **Relationship between Phosphate and Cholesterol in the Serum.**
    *Paul P. Carbone** *and Kathleen E. Roberts,* San Francisco. *page 67*
12. **Effect of Aortic Constriction on Experimental Atherosclerosis in Rabbits.**
    *David D. Snyder** *and Gilbert S. Campbell,** Oklahoma City. (Introduced by *Philip C. Johnson.*) *page 61*
13. **Histamine Metabolism in Human Disease.**
    *Gildon N. Beall** *and Paul P. VanArsdel, Jr.,* Seattle. *page 69*

THURSDAY, JANUARY 29

9:00 A.M.

14. **Bronchial Collapse in Pulmonary Emphysema: a Morphologic Study.**
    *Robert R. Wright,** San Francisco. (Introduced by *William Mandel.*) *page 84*
15. **Electrodes for Blood pO_2 and pCO_2 Determination.**
    *John W. Severinghaus,** San Francisco. (Introduced by *James Hopper, Jr.***) *page 83*
16. **Complete Inhibition of Erythocytic Carbonic Anhydrase in Vivo.**
    Clarence Collier, Loma Linda, California. *page 54*

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Advance Reports Submitted to the Annual Meeting of the

WESTERN SECTION

of the

American Federation for Clinical Research

Golden Bough Theater, Carmel, California
Wednesday and Thursday, Jauuary 28 and 29, 1959

BLOOD

The Influence of Exogenous Iron on Hemoglobin Formation in the Premature Infant

By *Denman Hammond and Arlene Murphy.* Department of Pediatrics, University of California, San Francisco.

Premature infants were randomly assigned to 1 of 2 groups, A and B, paired by birth weight. Group B was given 100 mg. iron intramuscularly over a period of 2 to 4 days, as soon as weight gain was consistent. Group A served as untreated controls. Hematologic studies were performed on all infants weekly during their nursery stay and monthly thereafter during clinic visits. No dietary restrictions were imposed, but medicinal iron was prohibited.

Hematologic and iron biochemical studies were performed on mothers of prematures on the 2nd or 3rd post-partum day and repeated 6 weeks later.

Sixty-six prematures were followed for periods from 1 to 18 months. Data on infants followed for at least 3 months were included in the results. No initial hematologic differences of statistical significance were found between mothers or infants. Group A had a mean gestational age 2 weeks longer than Group B, a mean birth weight greater by 200 Gm.

Hemoglobin concentrations fell in parallel during the 1st month of life. From the 1st to the 2nd month Group B had a slower rate of fall than Group A. By the 3rd month values had risen in both groups and Group B had a significantly higher hemoglobin—a difference persisting through the 1st year. Erythrocyte counts and hematocrits showed similar differences advantageous to Group B.

Total circulating hemoglobin mass calculations revealed Group B regained their birth hemoglobin mass by 3¼ months of age; Group A required 4½ months.

These results indicate that iron from exogenous sources may be utilized by the premature infant as early as the 2nd month and may be preferentially utilized for hemoglobin formation at a time when body iron stores are presumably not depleted.

Separation of the Effects of Potassium Depletion and Cold Storage on Erythrocyte Potassium Exchange

By *E. R. Borun.* Department of Medicine, UCLA Medical Center, and V. A. Center, Los Angeles.

This study demonstrates an increased rate

of potassium influx during incubation of previously cold-stored erythrocytes and relates this change to both intracellular potassium depletion and additional effects of storage.

Two portions of heparinized blood from 7 subjects were stored at 4 C. for 2 to 3 days with glucose and 2 volumes of isotonic phosphate buffer containing either 0 or 150 mEq./L. of potassium, respectively. Cold-stored and fresh specimens from the same subjects were washed and simultaneously incubated in a physiologic plasma-buffer mixture with $K^{42}Cl$ at 37 C. Aliquots removed at 3 intervals were centrifuged; plasma and cells were separated and analyzed for radioactivity, potassium and hemoglobin concentration. Potassium flux was calculated from formulas of Sheppard and Martin and of Tosteson.

The cells stored without added potassium, those stored with high potassium buffer, and fresh cells had potassium concentrations, respectively, of 88 ± 2 (mean ± S.E.), 99 ± 1 and 99 ± 1 mEq./L. at the time of incubation, and had influx rates of 3.8 ± 0.4, 2.6 ± 0.1 and 2.1 ± 0.1 mEq./L. cells/hour. The differences between flux rates are significant ($p < 0.01$).

A major portion of the increment in potassium influx in the cold-stored cells could thus be eliminated by preventing potassium depletion. The increment in flux secondary to potassium depletion cannot be explained on the basis of a decrease in the intracellular/extracellular concentration ratio since it occurred when extracellular potassium was lowered sufficiently to prevent a decrease in this ratio. Potassium depletion must activate some other mechanism responsible for regulating intracellular concentration. On the other hand, the residual increment in potassium influx observed in cold-stored cells with a normal intracellular potassium concentration must be due to an additional effect of the storage conditions per se, perhaps operating through the mechanism of increased membrane permeability.

Complete Inhibition of Erythrocytic Carbonic Anhydrase in Vivo

By *Clarence Collier*. Department of Physiology, College of Medical Evangelists, Loma Linda, California.

In vitro studies done elsewhere have indicated that it is unlikely that administration of acetazoleamide (Diamox) interferes significantly with RBC carbonic anhydrase, even in a dose much larger than usually given therapeutically. In these studies, the effect of graded intravenous dose of acetozoleamide was measured in dogs anesthetized with pentobarbital, paralyzed with succinylcholine and breathed at constant tidal volume with a Starling pump.

The end-tidal CO_2 tension was measured continuously with an infrared CO_2 analyzer. The arterial CO_2 tension was measured as frequently as every 2 minutes by use of an aerotonometer. The ratio of end-tidal or alveolar (A) to arterial (a) CO_2 tension was calculated for the control and experimental states. Acetazoleamide concentrations were measured by the method of Maren.

The control arterial CO_2 tension was usually about 30 mm. Hg, and the control A/a ratio ranged between 0.9 to 1.0. After graded doses of acetazoleamide, the A/a ratio dropped progressively but did not fall below 0.5 in spite of very large doses. The maximal effect was reached with an intravenous dose of 15–25 mg./Kg. The RBC concentration of acetazoleamide producing maximal effect ranged from 20–30 μg./cc. ($9–13 \times 10^{-5}$ molar). Higher concentrations of drug had no further effect.

If there had been no change in pulmonary blood flow or transit time in the pulmonary capillary, it must be assumed that complete carbonic anhydrase inhibition was produced with moderate doses of acetazoleamide. In the absence of carbonic anhydrase activity, very little bicarbonate CO_2 can be exchanged during the short transit time in the pulmonary capillary. The bulk of CO_2 exchange must be from dissolved CO_2 and carbamino-bound CO_2. Theoretical analysis indicates that these 2 means of transport are probably adequate to account for the exchange under the conditions of this experiment.

Observations on Red Cell Survival in Normal Adult Males following Splenectomy

By *Norman Ende and Armand P. Gelpi*, with the technical assistance of *James Castle*. V. A. Hospital, Nashville, Tennessee; San Leandro, California; and V. A. Hospital, San Francisco.

Chance observation of apparent prolonged survival of normal donor red cells in a splenectomized patient with a primary red cell defect prompted investigation of red cell survival in normal patients following splenectomy. The investigation was designed to determine if splenectomy enhances red cell survival in this group.

Seven patients with no known hematologic

defects were selected for red cell survival studies, using the Cr^{51} method of Necheles et al. (J. Lab. & Clin. Med. 42:358, 1953). Normal 50% red cell survival time is between 28–35 days. Each patient had routine hematologic studies including red cell osmotic fragility determinations and siderocyte (%) counts. Four normal individuals, spleens intact, were similarly studied. One splenectomized individual also received labeled, normal donor red cells.

Survival of labeled, auto-transfused red cells in the splenectomized patients varied within the following limits: 50% red cell survival from 30–44 days. The same variability occurred with the controls. The splenectomized individual transfused with normal donor cells had 50% red cell survival at 49 days. The splenectomized patients showed a slight leukocytosis, but not the decreased red cell osmotic fragility and increase in siderocytes said to occur following splenectomy.

Within the liimts of this study, there is no indication that splenectomy increases red cell survival in otherwise normal, adult males. The findings of normal red cell osmotic fragility and low siderocyte percentage are at variance with other published data in this regard.

Gross Hemoglobinuria following Intravenous Glycerin in the Human

By *Edward H. Storer and Joe Campbell.* Department of Surgery, University of Tennessee College of Medicine, Memphis.

Glycerin-containing solutions have been used as a storage medium for human erythrocytes at sub-zero temperatures. It has been thought necessary to remove the glycerin before administration of the cells to humans. Glycerin is also a major component of certain anhydrous fat emulsions currently being investigated for human intravenous use.

Little is known of the effects of i.v. glycerin in the human. One recent study reported that no hemoglobinuria occurred after intravenous administration of 50 Gm. glycerin as a 5% solution. However, gross hemoglobinuria has been noted after administration of fat emulsions containing 63 Gm. glycerin as a 12.6% solution.

Ninety-five infusions of 5% dextrose in water containing 3 different concentrations of glycerin were given to randomly selected hospital patients. Forty-seven infusions containing 63 Gm. glycerin as a 6.3% solution were given with no instances of hemoglobinuria. Eleven infusions containing 47.5 Gm. glycerin as a 9.5% solution were given with no hemoglobinuria. Thirty-seven infusions containing 63 Gm. glycerin as a 12.6% solution resulted in hemoglobinuria following 8 of the infusions—an incidence of 22%.

Therefore, since the same amount of glycerin was administered in the same length of time but in different concentrations, it would appear that the concentration of glycerin is the critical factor in the production of intravascular hemolysis.

The Relation of B_{12}-binding to the Uptake of Vitamin B_{12} by the Rat Liver Slice in Fractions from Human Gastric Juice

By *P. C. Johnson, T. D. Driscoll and J. B. Faulkner.* Radioisotope Service, V. A. Hospital, and Department of Medicine, University of Oklahoma, Oklahoma City.

Gastric intrinsic factor activity is poorly correlated with vitamin B_{12}-binding activity, yet it is not known whether binding is necessary for intrinsic factor activity. Gastric juice when chromatographed on ambrolite IRC-50 Resin by our modification of the method includes at least 5 protein-containing fractions characterized by more or less well-defined peaks. Peak No. 1 contains most all of the polysaccharides including "A" substance. Peak No. 2 contains pepsin. Peak No. 4 contains a newly described non-pepsin proteolytic enzyme called gastricsin, and No. 3 and 5 are protein peaks without established physiologic activity.

We have used this method to analyze dialyzed gastric juice specimens from 3 normal subjects. Cobalt-60 labelled vitamin B_{12} binding was present only in peaks 1, 4 and 5. The percentage of the total binding activity (dialysis method) was for each peak: No. 1, 33%; No. 4, 18%; and No. 5, 49%. Inactivation of the proteolytic enzymes destroyed the binding activity of peak No. 4.

Using the rat liver slice technic as a measure of intrinsic factor activity, we have found that each peak enhanced the uptake of vitamin B_{12}. Enzyme inactivation did not change this uptake. The effluent from the column between the protein peaks showed no uptake. Chromatography of a gastric juice (diabetic patient) containing free acid and no proteolytic activity did not bind vitamin B_{12} but did promote the uptake by the liver slice. This patient had a normal liver uptake of vitamin B_{12}. Gastric juice

from a pernicious anemia patient showed binding, but no increase in uptake by the rat liver slice.

The evidence suggests 3 substances in human gastric juice which bind vitamin B_{12} and 4 substances that promote the uptake of radioactive B_{12} by the rat liver slice. Destroying the binding activity did not change the uptake by the liver slices.

The in Vitro Incorporation of Nucleic Acid Precursors by Normal and Leukemic Human Leukocytes

By *J. L. Scott, G. E. Cartwright and M. M. Wintrobe*. Department of Internal Medicine, University of Utah College of Medicine, Salt Lake City.

Preliminary studies of the in vitro incorporation of labelled nucleic acid precursors by human leukocytes have been made for the purpose of clarifying the antileukemic action of therapeutic antimetabolites.

Blood leukocytes were separated by dextran sedimentation of the RBC and centrifugation; resuspension in 50% normal serum—Hanks' solution; and incubation at 37 C. for 4 and 8 hours with adenine-8-C^{14}, glycine-1,2-C^{14} or formate-C^{14}. Following incubation the cell nucleic acids were isolated. In some experiments the nucleic acids were fractionated into DNA and RNA by mild alkaline hydrolysis; in others the soluble nucleotide co-enzymes were also recovered. After acid hydrolysis the constituent bases were isolated by paper chromatography and their specific activities determined.

Normal, chronic myelocytic, chronic lymphocytic and acute leukemic cells incorporated adenine-C^{14} into the total nucleic acid fraction and converted labelled adenine to guanine. The specific activity of the RNA purines was 10–20 times that of the DNA purines in both chronic myelocytic and acute leukemic cells. With glycine-C^{14} inconstant *de novo* purine synthesis was observed in chronic myelocytic leukemic cells, but not in normal, chronic lymphocytic or acute leukemic cells. No effect of 6-mercaptopurine or its riboside was observed on the incorporation of labelled adenine, its conversion to guanine or the *de novo* synthesis of purines from glycine.

Formate-C^{14} was incorporated into both the nucleic acid purines and the thymine of acute leukemic cells. Amethopterin inhibited this incorporation.

Thus, isolated circulating leukocytes can actively incorporate various purine and pyrimidine precursors in vitro. This technic appears to have promise in investigating the mechanism of action of 6-mercaptopurine and other antimetabolites and the processes by which therapeutic resistance to these agents develops.

Observations on the "Pseudo"-Pelger Phenomenon

By *Edward Shanbrom, Zeola Collins and Sherwood Miller*. Department of Hematology, City of Hope Medical Center, Duarte, California.

The Pelger-Hüet anomaly is an hereditary abnormality of granulocytes characterized by failure of the nucleus to segment beyond the "band" stage. The nuclei may appear rodlike, rounded or bilobed ("spectacle" cells). "Pseudo"-Pelger leukocytes have been sporadically reported in association with other diseases, notably leukemia. Interest in the phenomenon was stimulated by the appearance of these abnormal cells during antimetabolic therapy of leukemic patients.

The marrow and blood of 70 patients with various leukocyte disorders were carefully reviewed. Differential counts of mature neutrophilic cells were performed on blood smears of those patients exhibiting the "pseudo"-Pelger anomaly. Abnormalities of the granulocytes were observed in 19 patients. Of these, there were 12 cases of chronic granulocytic leukemia, 3 of acute granulocytic leukemia, 3 of myeloproliferative syndrome and 1 acute lymphocytic leukemia.

The relatively common occurrence of "pseudo"-Pelger leukocytes in this series is evidence that the abnormality is not as rare as is generally thought. The frequency with which the phenomonen appears in leukemia suggests a relationship to other abnormal leukocytes which may appear in the course of leukemia, such as micromyeloblasts, Rieder cells, Türck cells and Ferrata cells. The fact that "pseudo"-Pelger granulocytes have been observed to develop during treatment of leukemia and can be induced experimentally in rabbits by intravenous administration of colchicine is evidence that chemotherapeutic agents may contribute to the formation of the abnormal cells. It is suggested that "pseudo"-Pelger-Hüet leukocytes and other abnormal cells seen in leukemia represent asynchronous maturation between the nucleus and cytoplasm, possibly due to an enzymatic derangement of nucleic acid metabolism.

Familial Sex-linked Thrombocytopenia: Response to Splenectomy

By *Charles Brubaker and Denman Hammond.* Division of Hematology, Childrens Hospital of Los Angeles, and Department of Pediatrics, University of Southern California School of Medicine.

A new, familial syndrome characterized by thrombocytopenia, impaired resistance to infection and eczema was first reported in 1954 by Aldrich et al. Eight such cases, all involving male children, are now described in the literature. The disorder appears to be sex-linked, recessive and unresponsive to various therapeutic measures. Most cases have expired during early childhood from hemorrhage or overwhelming infections. Splenectomy in 5 cases was reported to produce little or no hematologic benefit and possibly to increase the patients' susceptibility to infection. This report concerns the clinical and hematologic studies on 3 additional cases that were improved following splenectomy.

Two patients had an older, male sibling and the 3rd case a maternal uncle who expired in childhood of a similar clinical syndrome. Each of the cases had (1) onset of symptoms during the 1st year of life, (2) marked thrombocytopenia with hemorrhagic manifestations, (3) eczematoid dermatitis, and (4) frequent infections, especially of the skin, upper respiratory tract and middle ears. Although bone marrow aspirates showed reduced numbers of megakaryocytes and splenomegaly was not present, splenectomy resulted in correction of the thrombocytopenia and clinical improvement in all 3 cases.

Literature review indicates that this syndrome has been confused with idiopathic thrombocytopenic purpura, from which it should be differentiated. This confusion may have contributed to the recent suspicion that splenectomy in infancy may predispose to overwhelming infection. These patients have impaired resistance to infection prior to splenectomy. Studies of serum proteins and immune mechanisms of these patients have not revealed quantitative or qualitative abnormalities to date.

The Significance of the para-Toluene Sulfonic Acid Precipitation Test in Blood Dyscrasias

By *Helmut Haar, Edward Shanbrom and Mary Jane Patch.* Department of Hematology, City of Hope Medical Center, Duarte, California.

The p-toluene sulfonic acid precipitation test, originally suggested as a specific diagnostic aid in systemic lupus erythematosus (SLE) by Jones and Thompson, has also been positive in other diseases. However, the test has not been studied extensively in hematologic disorders. Ninety-eight tests were performed in 83 patients with various blood dyscrasias. Thirty-six specimens were considered significantly positive. Of these, 14 were multiple myeloma, 4 leukemias, 3 Hodgkin's disease, 3 other lymphomas, 2 myeloproliferative syndromes and 2 undiagnosed anemias. Positive tests were also found in 3 other patients with postnecrotic cirrhosis of the liver, sarcoidosis and coccidiomycosis. Nine tests were done in 8 patients with SLE and only 2 were considered positive.

Additional studies of serum proteins were performed in patients with positive precipitation tests. An attempt was made to correlate positive serums with the A/G ratio, cephalin flocculation, thymol turbidity, cryoglobulin determinations and serum electrophoretic patterns. In multiple myeloma the test was strongly positive in 4 patients with typical gamma-globulin peaks, and in 3 cases with beta-globulin peaks. There was no precipitation in the serum of a patient exhibiting an alpha^2 peak. Of the chronic leukemias and lymphomas who reacted positively, 6 showed elevation of the gamma fraction. The patients with hepatic cirrhosis, coccidiomycosis, sarcoidosis and the 2 positive-reacting SLE cases, all had high gamma-globulin values. On the other hand, a number of patients with elevation of the gamma fractions failed to react positively. There was no distinct correlation with cephalin flocculation, thymol turbidity or cryoglobulin tests.

These studies indicate that the p-toluene sulfonic acid precipitation test is not specific for systemic lupus erythematosus. It may, however, be of value as a simple diagnostic aid in the detection of dysproteinemic states.

Uracil-Mustard, a New Chemotherapeutic Agent: Preliminary Clinical Evaluation in Hematologic Disorders

By *Edward Shanbrom, Sherwood Miller and Helmut Haar.* Department of Hematology, City of Hope Medical Center, Duarte, California.

Seventy patients with various hematologic neoplasias were treated with a new oral alkylating agent, U-8344 (5-bis [2'-chloroethyl] aminouracil, "uracil-mustard").

Of these, there were 23 cases of Hodgkin's disease, 8 lymphosarcoma, 2 giant follicular lymphoma, 14 chronic granulocytic leukemia, 11 chronic lymphocytic leukemia, 4 polycythemia and 4 multiple myeloma. Most patients had received other treatment and many were resistant to other chemotherapeutic agents. All had measurable objective evidence of the disease process. The drug was administered orally in doses of .01 to .05 mg./Kg./day in both divided and single doses. Intermittent and continuous regimens were utilized, and the majority of patients were on maintenance therapy for periods of up to 6 months.

U-8344 appears to exhibit a wide spectrum of therapeutic activity in lymphomas and leukemias. Objective criteria for beneficial response were reduction of lymphadenopathy or hepatosplenomegaly, subsidence of fever, weight gain, decrease in elevated leukocyte counts and rise in hematocrit. Good responses were noted in chronic granulocytic and lymphocytic leukemia, lymphosarcoma and giant follicular lymphoma. Good results were also observed in Hodgkin's disease, although in some patients the remissions were of short duration; however, all these patients had far-advanced disease and were resistant to other therapeutic agents. Subjective improvement was seen in 3 myeloma patients. The drug appears to act slowly in many patients and improvement may not be seen for 9 to 12 weeks.

Toxic effects are mild and infrequent. Nausea, vomiting and diarrhea occurred with larger doses (.03 to .05 mg./Kg.) but subsided when the dose was reduced. Bone marrow depression developed in some patients but was readily reversible after discontinuance of the drug.

BLOOD PROTEINS

Quantitation of Gamma Globulin in Human Serum by Immunoprecipitation

By *Beach Barrett, Wade Volwiler and Patricia Ann Wood.* Department of Medicine, University of Washington School of Medicine, Seattle.

The detection and follow-up of patients with agammaglobulinemia and hypogammaglobulinemia depends upon a precise determination of the level of gamma globulin in serum specimens. The immunoprecipitation technic was studied to determine its suitability for accurately measuring low levels of gamma globulin.

Antisera to human gamma globulin were prepared in rabbits. These antisera were tested for the presence of cross-reacting antibodies using 3 different agar technics (Oudin tube, Ouchterlony plate and Grabar-Williams' immunoelectrophoresis) and by detailed precipitation with agammaglobulinemic serum. Cross-reacting antibodies were precipitated from the antisera by adding small amounts of agammaglobulinemic human serum.

The purified antisera were standardized against solutions of human gamma globulin and were used to quantitate the level of gamma globulin in a number of normal and hypogammaglobulinemic sera.

Normal sera were found to contain from 1100 to 2200 mg.% of gamma globulin by immunoprecipitation as compared to a range of 800 to 1900 mg.% by scanning paper electrophoretic strips. The lower value obtained by electrophoresis is probably due to the fact that this method ignores protein which migrates in the α_2 and β range but which reacts immunologically as a gamma globulin.

Sera containing 2 to 200 mg.% of gamma globulin were quantitated repeatedly by immunoprecipitation. The coefficient of variation ranged from 2 to 7 in quintuplicate determination. Such precise quantitation of hypogammaglobulinemic sera by electrophoretic methods is impossible because of an inherent error on the order of ± 100 mg.%. Electrophoresis is a valuable screening procedure which will identify hypogammaglobulinemic sera for further quantitative study.

It is concluded that the immunoprecipitation method is an accurate and practical means of diagnosing and studying patients with abnormally low levels of circulating gamma globulin.

Bone Marrow Transplant in Agammaglobulinemia

By *Casimir A. Domz.* Sansum Medical Clinic Research Foundation, Santa Barbara, California.

Bone marrow homotransplantation is technically simpler, and could provide agammaglobulinemic (AGG) patients with antibody-producing tissue (lymphocyte-plasma cell system) similar

to that provided by lymph node homotransplants previously reported.

A patient with adult-acquired AGG was infused with 70 ml. of bone marrow from an ABO-compatible donor. The donor was not prestimulated antigenically to increase the plasma cell count or gamma globulin production.

Serum protein electrophoresis at monthly intervals showed a progressive increase in concentration of gamma globulin (GG) from 0.3 to 0.6 Gm.%. At 6 months the CG concentration reached the lower limit of normal, and the patient's monthly injections of gamma globulin were withheld. This was not followed by the appearance of infection clinically. At the end of the 8th month, however, the GG concentration returned to its previous level.

Monthly bone marrow aspirates showed no rise in plasma cell count. Antigenic challenge with typhoid-paratyphoid vaccine showed no rise in titer by clinical agglutination methods, or by Boyden's hemagglutination technic. Serum complement, originally above normal, fell abruptly to subnormal levels and gradually rose in 8 months to its original high value.

Spontaneous remission in adult-acquired AGG does not occur. An increase in GG level by cumulation from the previous monthly injections seems unlikely, in that electrophoretic studies (each done just prior to the monthly GG injection) had shown a constant serum GG level during 18 months preceding bone marrow transplantation. While the rise in this patient's GG level cannot with certainty be ascribed to the bone marrow homotransplant, the evidence suggests this possibility.

Graft survival was apparently only temporary, the duration of effect being similar to that achieved with lymph node homotransplants. Homotransplants of skin survive indefinitely (more than 3 years) in AGG patients, but it seems likely that transplants of antibody-producing tissue will not be effective unless the graft is protected by a diffusion chamber.

CARDIOVASCULAR SYSTEM

A Simplified Technic for the Visualization of the Conduction System of the Human Heart

By *Herman N. Uhley and Laurence M. Rivkin.* Harold Brunn Institute and Department of Surgery, Mount Zion Hospital and Medical Center, San Francisco.

The A-V bundle and its ramifications in the human heart are extremely difficult to visualize, and the possibility of its accurate dissection and, indeed, even its existence have variously been questioned. A simple method for visualizing the conduction system, therefore, would be of invaluable aid in its accurate identification. Although histologists long have been familiar with the use of Lugol's solution for staining glycogen, only recently has it been used to identify the glycogen-rich cardiac conduction system in dogs.

The technic involved consists simply of direct application of aqueous Lugol's solution, with cotton swabs, to the endocardial surface of the human heart. If the glycogen stores are not depleted, the bundle appears as a dark-stained band with ramifications on the septal wall. This provides an exceedingly simple means for gross visualization of the A-V bundle system. The technic has obvious applicability in electrocardiographic and in clinico-pathologic correlations.

Coronary Blood Flow Determined by Surface-counting Technic and Ratio Formula

By *G. Sevelius and P. C. Johnson.* Radioisotope Service, V. A. Hospital, and Department of Medicine, University of Oklahoma, Oklahoma City.

Prinzmetal and others have shown that an estimate of cardiac output can be obtained by recording the time activity curve of intravenously injected radioiodinated serum albumin through the heart with a scintillation tube placed on the body surface over the heart. By using a high frequency response in our rate meter we have found that an extra peak is present on the downslope of the curve of the left ventricle. This peak appears simultaneously with the peripheral circulation as measured by the appearance of the radioactivity in the carotid artery. When the detector is placed over the base of the heart this peripheral circulation peak is dominated by the coronary blood flow. A formula for calculating blood flow from the peripheral circulation peak was developed in our laboratory in connection

with work on renal blood flow. This formula was used for coronary blood flow as follows:

$$\text{coronary blood flow} = \text{cardiac output} \div \frac{A^2_h}{A_c^2} \times \frac{T_c}{T_h}$$

where: A = areas of the curve; T = time of first passage of radioactivity; h = heart; c = coronary.

The coronary blood flow in different clinical diseases and age groups was determined. The mean ± standard error of the mean in 19 normal male patients was 306 ± 18 cc./min. In 9 male patients with coronary insufficiency, the mean coronary blood flow was 162 ± 9 cc./min. ($P < .001$).

The mean ages of these 2 groups were 49 ± 14 and 50 ± 12 years, respectively. The mean weights were 152 ± 5 and 150 ± 4 pounds for the coronary group. Coronary blood flow calculated on a weight basis was 2.0 ± 0.5 and 1.1 ± 0.2 cc./min. per pound weight. Using this technic, 65 mg. nitroglycerine increased the coronary blood flow in a normal 65%.

Autoregulation of Coronary Blood Flow in the Dog Heart

By *Malcolm E. Fishback, Leland Burnett and Allen M. Scher.* Department of Physiology and Biophysics, School of Medicine, University of Washington, Seattle.

Experiments were designed to determine if resistance to coronary blood flow is autoregulated, i.e., if flow is altered by some intrinsic mechanism independent of external nervous and humoral influences. Such a phenomenon has been described in kidney and skeletal muscle.

Hearts from mongrel dogs were perfused with blood from anesthetized donor dogs. Simultaneous records of perfusion pressure (which could be altered by a pump), inflow, outflow and heart and coronary sinus hematocrit were taken electronically.

In 10 preparations, it was found that when flow was plotted against perfusion pressure as an independent variable, a sigmoid curve was produced. Over a range of pressures, usually 60 to 130 mm. Hg, the flow rate increment diminished relative to pressure increment. Weight of the perfused heart increased linearly with increased perfusion pressure, and coronary sinus hematocrit increased and decreased transiently with pressure increase or decrease, respectively.

It is concluded that over a certain pressure limit, coronary flow is autoregulated in both the beating and fibrillating perfused canine heart.

Calcific Coronary Arteries

By *David H. Blankenhorn.* University of Southern California School of Medicine, Los Angeles.

A study has been performed to determine the incidence and significance of calcific lesions in the human heart. Postmortem radiographs of 80 unselected patients 17 to 95 years of age demonstrate that the most common site of calcification in the heart is in the coronary arteries, and that coronary calcification can be differentiated from other intracardiac calcification by location and appearance.

Microscopic examination of the coronary arteries from these hearts indicates that radiologically visible calcification of the coronary invariably occurs in atheromatous plaques in the adult. In this regard the present study is in accord with the literature on coronary calcification. In addition, certain coronary arteries with occlusive atheromatous plaques leading to ischemic heart disease can be distinguished by their radiographic pattern of calcification from others in which extreme dilation compensates for calcific atheromatosis, thereby sparing the myocardium.

The Removal of Intravenously Injected Triglycerides from the Plasma of Young Men with Arteriosclerotic Heart Disease

By *C. W. Smith and P. C. Johnson.* Radioisotope Service, V. A. Hospital, and Department of Medicine, University of Oklahoma, Oklahoma City.

Relationships between atherosclerosis and abnormal lipid metabolism have been widely accepted, although incompletely defined. Determination of the rate of triglyceride removal from the plasma after a fat meal is unsatisfactory due to the relatively slow rate of absorption from the gastrointestinal tract.

Forty cc. of Lipomul, containing 6.0 Gm. of triglycerides, was given intravenously to 16 control patients and to 9 patients with proven myocardial infarctions. All patients were less than 45 years of age. Plasma samples were obtained fasting and at 10, 20 and 30 minutes following the injection. In addition, an aliquot of each 10-minute sample was incubated at 37 C. for 2 hours as a measure of in vitro clearing factor activity, a technic by which we have demonstrated post-heparin clearing factor activity by an in vitro decrease of triglycerides. Triglycerides were determined by the method of Van Handel and Zilversmit. The rate of removal was

determined by plotting the triglyceride excess semilogarithmically against time.

The mean total fasting plasma triglycerides of the patients with arteriosclerotic heart disease was significantly higher ($p < .002$) than that of the control patients. The triglyceride excess was removed from the plasma at the same rate in both groups. In both groups the mean total triglyceride in the incubated aliquot of the 10-minute sample failed to show in vitro clearing factor activity since the value was slightly but significantly higher ($p < .001$) than the unincubated aliquot.

It is concluded that the triglyceride excess during the first 30 minutes after an intravenous load is removed from the plasma at normal rates in young men with arteriosclerotic heart disease. Their fasting plasma triglycerides are higher than normal. Intravascular clearing factor activity is not an important means of removing this triglyceride load.

Effect of Aortic Constriction on Experimental Atherosclerosis in Rabbits

By *David D. Snyder and Gilbert S. Campbell.* Department of Surgery, University of Oklahoma Medical Center, and Oklahoma City V. A. Hospital.

Surgical relief of segmental arterial occlusion in patients may be followed by progressive atherosclerosis in the distal arterial tree. Therefore, we tested whether aortic constriction in rabbits might alter the production of distal atherosclerosis.

Thirty adult female rabbits, fed a high cholesterol diet, were subdivided into 3 groups: (1) 10 non-operated controls; (2) 10 sympathectomized rabbits (bilateral lumbar sympathectomy and periaortic adventitial stripping from the renal arteries to the aortic bifurcation) without aortic constriction; (3) 10 sympathectomized rabbits with infra-renal aortic constriction. After 3 months, femoral artery blood pressure tracings were obtained in each rabbit and the rabbits were sacrificed. The non-operated rabbits demonstrated almost solid coalescence of atherosclerotic plaques in the thoracic aorta but only occasional plaques distally. As Murphy has demonstrated, the sympathectomized rabbits showed severe involvement of the lumbar aorta and iliac arteries, as well as the thoracic aorta. Sympathectomized rabbits with aortic constriction demonstrated severe atherosclerosis in the thoracic and lumbar aorta down to the level of constriction, but only occasional or rare plaques below this level.

Prior to sacrificing these rabbits, direct pressure measurements above and below the aortic constriction were obtained. All 10 rabbits with aortic constriction demonstrated a marked diminution of pulse pressure below the constriction.

In rabbits with sympathectomy and aortic constriction the denervated aorta was subjected to the full systolic thrust above the constriction, but this force was considerably damped below that area. It has been suggested by Page that atherogenesis is due to the accumulation of substances filtered from plasma through the intima by the lateral pressure. Therefore, in this study decreased lateral pressure below the level of aortic constriction may explain diminished atherogenesis as a result of decreased filtration of lipemic plasma through the intima.

The Temperature of Venous Blood in the Extremities and Its Relationship to the Clotting Process

By *Edward Rubenstein and Arthur Lack.* Departments of Clinical Physiology and Pathology, Community Hospital of San Mateo County, and Department of Medicine, Stanford University School of Medicine, San Francisco.

This study was undertaken to establish the normal temperatures of venous blood in the extremities and to determine the effect of these temperatures on blood clotting.

Temperatures were measured under ordinary room conditions by means of needle thermistors. In groups of 20 normal adults the following mean temperatures and ranges were found: greater saphenous veins (lower 3rd of leg), 30.0 C. (S.E. ± 0.419), 27.2 C. to 32.8 C.; antecubital veins, 34.5 C. (S.E. ± 0.256), 33.2 C. to 37.4 C.; deep gastrocnemius muscle, 35.4 C. (S.E. ± 0.259), 32.8 C. to 37.4 C. In groups of 20 afebrile hospitalized adults confined to bed for more than 72 hours, these mean temperatures and ranges were found: greater saphenous veins, 29.6 C. (S.E. ± 0.452), 25.4 C. to 33.2 C.; antecubital veins, 34.4 C. (S.E. ± 0.247), 32.4 C. to 37.1 C.; deep gastrocnemius muscle, 33.5 C. (S.E. ± 0.270), 31.6 C. to 35.4 C.

In the range from 22 C. to 37 C. the following linear negative temperature coefficients were found: one-stage prothrombin time, 0.74 seconds/°C.; Lee-White clotting time, 7%/°C.; whole blood viscosity, 0.15 centipoise/°C.

Clot fragility was estimated by determining

the weight of a mercury-filled graduated cylinder which could be supported by a disc-shaped clot formed in a beaker. Twenty paired blood specimens were allowed to clot for 18 minutes at 37 C. and at room temperature. The weight which forced the base of the cylinder through the clot was higher for each of the warmer clots, $p < 0.001$.

Conclusions: (1) Superficial venous leg temperatures are relatively cool, about 30 C.; in the arms they are approximately 34.5 C. (2) Inactivity is associated with lowering of leg muscle temperature. (3) Reducing temperature from 37 C. retards the clotting mechansim but increases blood viscosity and results in fragile clots. (4) These low temperature effects may be of clinical importance in thrombo-embolism and may help explain the high incidence of emboli arising from the legs as compared with the arms or the rest of the body.

Cardiovascular Involvement in Lupus Erythematosus

By *Martin A. Shearn.* Department of Medicine, Permanente Medical Group, Kaiser Foundation Hospital, Oakland, California.

Eighty-three patients with systemic lupus erythematosus were analyzed with respect to cardiovascular abnormalities. Three fourths of the patients had some finding referable to the heart. Systolic murmurs appeared in 70%, but did not correlate with endocardial disease. They were of greatest intensity in the presence of profound anemia. Diastolic murmurs were noted in only 4 patients, in 2 of whom atypical verrucous endocarditis of the mitral valve was found at necropsy. Hypertension was present in 27 (33%) of the patients; of these, 7 had the nephrotic syndrome, 13 manifested nitrogen retention, and all exhibited some abnormality of urine sediment. Congestive heart failure occurred in 15 patients, of whom 11 were hypertensive, 11 had hemoglobin concentrations below 9 Gm./100 ml., and 12 (of 14) showed electrocardiographic evidence of myocardial involvement. Electrocardiograms of 73 patients were available. Abnormalities were present in 45, the most frequent being inversion of the T waves. Arrhythmias were infrequent. Myocardial infarction was not seen. Pericarditis was found in about ⅓ of the patients. Six had pericardial effusion, but in only 1 did cardiac tamponade occur.

ENDOCRINES AND METABOLISM

Relationship of Nitrogen-retaining and Diabetogenic Activity of Growth Hormone Preparations in Man

By *Rex Bigler, Ernest Gold, Vincent C. Di Raimondo, Richard J. Havel, Choh Hao Li and Peter H. Forsham.* Metabolic Unit for Research in Arthritis and Allied Diseases and Department of Medicine, University of California School of Medicine, San Francisco, and Hormone Research Laboratory, Berkeley.

Human growth hormone was administered to a pancreatectomized subject on a constant diet stabilized with 30 units NPN insulin and 30 mg. of hydrocortisone daily, so that less than 4 Gm./day of urinary glucose were excreted. When placed on human growth hormone (Li), 5 mg. i.m. every 6 hours, there was a mean nitrogen retention of 3.5 Gm. daily with a concomitant rise in daily fasting blood sugars from an average of 134 mg. (before treatment) to 365 mg. and an increase in glycosuria ranging from 5 to 25 Gm. daily. There was no ketonuria despite an increase in unesterified fatty acids in the plasma.

This suggests a peripheral diabetogenic effect of human growth hormone, since pancreatic function was fixed in this pancreatectomized subject and adrenal cortical activity could not vary appreciably on 30 mg. of hydrocortisone daily.

Metabolic Activity of 3:5:3′-Triiodothyropropionic Acid in Hypothyroid and Euthyroid Patients

By *Marvin L. Sachs and Walter L. Arons.* Endocrine and Vascular Sections, Department of Medicine, Hospital of the University of Pennsylvania, Philadelphia.

The metabolic activity of 3:5:3′-triiodothyropropionic acid (Triprop) was evaluated in 6 euthyroid patients with goiters and in 5 hypothyroid patients. Symptoms, signs, BMR, PBI, I^{131} uptake and serum cholesterol, phospholipids and total esterified fatty acids were followed.

Hypothyroid patients brought to euthyroid status by Triprop were maintained by daily doses

of 3–6 mg. euthyroidism being associated with PBI levels elevated to 8–10 μg.%. Thus, potency and possibly protein-binding characteristics differ from those of thyroxine and triiodothyronine. A single oral dose of 20 mg. of Triprop, though causing no acute changes in signs or symptoms in a hypothyroid patient, elevated the PBI to 20 μg.% within 2 hours, with decrease to half the peak PBI level within 36 hours and to baseline level within 4–5 days. This dose elevated the BMR from the hypothyroid to the euthyroid range within 12 hours, with return to initial values within 72 hours.

In euthyroid patients given 1–6 mg. of Triprop daily, the PBI rose to 4.4–11.4 μg.%, increasing directly with the dosage. Within this dosage range, however, there was no consistent change in BMR, the values remaining approximately constant or rising slightly within the euthyroid range. Triprop was capable of decreasing the size of nodular and diffuse goiters and of depressing I^{131} uptake.

Serum lipids were depressed by Triprop in every subject, cholesterols falling 11–70% in the hypothyroid group and 10–28% in the euthyroid (and normocholesterolemic) group, the decrease varying directly with the initial cholesterol level. In 2 euthyroid patients followed for more than 6 months the lipid depression was not completely sustained.

Triprop is a potent compound for replacement therapy in myxedema and for depressing serum lipids in both hypothyroid and euthyroid patients. The interrelationships of dosage, clinical effects, PBI, BMR and serum lipids show some differences, at least quantitative, between Triprop and other thyroactive substances.

The Acute Antithyroid Effect of Cobalt Chloride

By *Bernhard G. Anderson*. Orange County General Hospital, Orange, California.

This study was designed to investigate the acute effects of cobalt chloride on the rate of thyroidal accumulation of radioactive iodine in man. Eleven euthyroid patients were given tracer doses of I^{131} and the thyroidal uptake of I^{131} was determined hourly for 6 hours. About 3 hours after the administration of the I^{131}, cobalt chloride ($CoCl_2 \cdot 6H_2O$, 1% solution) was given orally in doses of 80 mg. to 120 mg. Two additional patients were given 80 mg. and 100 mg., respectively, of cobalt chloride 1 hour before the administration of a tracer dose of I^{131}, and the thyroidal radioactivity was measured at ½ to 1-hour intervals, for 6 hours. The % of I^{131} accumulated by the thyroid was plotted against the square root of the elapsed time.

During the 3-hour observation period following the ingestion of cobalt chloride, the uptake of I^{131} was markedly inhibited in 3 patients, moderately inhibited in 3 patients and was not significantly altered in 5 patients. In the 2 patients who received cobalt chloride 1 hour before the I^{131} tracer dose, a prompt accumulation of I^{131} in the thyroid occurred within 30 minutes, and there was slight or no further increase in thyroidal I^{131} for 3 to 4 hours, followed by resumption of I^{131} uptake at a normal rate.

The results indicate that cobalt chloride in single oral doses of 80 to 120 mg. interferes with the thyroidal uptake of I^{131} in some subjects, but not in others. The pattern of the I^{131} accumulation rate suggests that cobalt chloride does not impair the iodide-concentrating mechanism but inhibits the organic binding of iodine by the thyroid.

Studies of Serum Insulin-like Activity in a Case of Severe Insulin Resistance

By *Paul M. Beigelman*. Department of Medicine, University of Southern California, and Los Angeles County Hospital, Los Angeles.

A 68-year-old female diabetic patient, with known diabetes of 11 years' duration, was admitted to the Los Angeles County Hospital in severe diabetic coma and expired 55 hours later. During this period, the blood sugar declined from 1200 to 472 in response to a total insulin dosage of nearly 100,000 units. In the meantime, the serum bicarbonate rose from <5 to 18. Elevated serum amylase levels were present throughout the course, but necropsy revealed no gross evidence of pancreatitis and no major gross pathologic change which could readily explain the death.

A method of insulin bioassay, utilizing glucose uptake by rat epidydimal adipose tissue, has been developed which is sensitive to as little as 10 micro-units (1/100,000 unit) of insulin per ml. Various dilutions of serum specimens from this case were tested by this technic. The 1st serum specimen was obtained 15 minutes following administration of 8,000 units of insulin i.v., a total cumulative dose of nearly 48,000 units in 40 hours having been given by then. A 2nd specimen was obtained 2 hours later, with

no intervening insulin treatment. Preliminary studies by this insulin bioassay technic indicate definite insulin-like activity at 1/100 dilutions of both sera—some activity at a 1/1000 dilution of the 1st serum specimen and little or no insulin-like activity at a 1/1000 dilution of the second serum specimen.

The Effects of 2 Synthetic Estrogens on Hypercholesterolemia Accompanying Diabetes Mellitus

By *Burt Cochran, Jr. and William W. H. Pote, Jr.* Medical Services, White Memorial and Los Angeles County Hospitals, and Department of Medicine, College of Medical Evangelists, Los Angeles.

In spite of certain presumably anti-atherogenic effects on plasma lipids, the clinical use of estrogens has been limited by the frequency of side effects, particularly gastrointestinal, uterine and breast. A "non-estrogenic estrogen" retaining potent lipid effects would seem the ideal agent.

This favorable ambivalence has been reported by Cook and Edgren from studies in cockerels and rodents for 2 synthetic substituted estrones: (1) 16-alpha-chlorestrone-3-methyl ether and (2) 1,4-dihydroestrone-3-methyl ether.

Thirty-four adult diabetic out-patients, representing a cross section of clinic-type problems, were selected for the presence of hypercholesterolemia independent of their level of diabetic control. Ages ranged from 37 to 80 years. There were 9 males and 3 premenopausal females. Diabetic care included insulin, tolbutamide and diet alone. During a minimum 3-month observation period baseline studies were obtained. No attempt at changing the subjects' diabetic control was made unless dictated by general clinic policy.

Serial studies included a hemogram, urinalysis, BUN, alkaline phosphatase, thymol turbidity, cephalin flocculation and serum glutamic oxalacetic transaminase. On each clinic visit a fasting blood sugar and serum cholesterol were obtained. Lipoproteins by paper electrophoresis and EKG's were followed intermittently.

16-Alpha-chlorestrone-3-methyl ether in stepwise increments (5 to 20 mg.) to 14 subjects effected an average decline of cholesterol 36% of the control level in 2 to 4 weeks (range 16–51%); average fall was 129 mg.% (range 45–190 mg.%). An increase in alpha lipoproteins preceded the major decline.

Each synthetic was administered on this plan and also in lower doses (up to 38 weeks). The latter infrequently achieved the above degree of cholesterol response. The initial drop of cholesterol was proportional to control hypercholesterolemia and to dosage used.

Estrogenic side effects were a common limiting factor, especially at higher dosages, and included mild to moderate mastalgia, gynecomastia, gastrointestinal distress, malaise, lassitude and both "break-through" and "withdrawal" uterine bleeding.

Studies of Pituitary Adrenocortical Relationships with an 11-Beta Hydroxylase Inhibitor of the Adrenal Cortex

By *Ernest M. Gold, Rex Bigler, Stanley Newman, Marielena Angers and Vincent C. DiRaimondo.* Metabolic Unit for Research in Arthritis and Allied Diseases and Department of Medicine, University of California School of Medicine, San Francisco.

Preliminary studies with 2-methyl-1, 2-bis-3 (pyridyl)-1-propanone (Ciba SU-4885) indicate marked interference with synthesis of cortisol by the adrenal cortex. Pituitary secretion of ACTH is consequently increased. Resultant stimulation of the inhibited adrenal cortex results in the rise of abnormal corticoid precursors lacking oxygenation in the 11 position of the steroid nucleus. Urine and blood samples collected at intervals before, during and after intravenous administration of SU-4885 were analyzed for Porter-Silber chromogens, 17-ketogenic steroids, pregnanetriol, 17–11 oxy to 17–11 desoxy corticoid ratios by Bush chromotography and 17-ketosteroid fractionation.

Subjects with normal adrenal function and selected endocrine patients were studied. Porter-Silber chromogens both in blood and urine increase rapidly within 2 hours after the beginning of an intravenous infusion of 800 mg. of SU-4885 over a 4-hour period. The peak of this rise occurs at 6 hours after the completion of the infusion and amounts to 2 to 3 times the baseline levels both in blood and urine. Within 8 hours after the end of the infusion, baseline values are once again approached.

A more precise indicator of altered adrenal cortical synthesis is the change in ratio of C21O

to $C21O_4$ corticosteroids from 3/1 to 1/1. Changes in pregnanetriol secretion were also followed.

Patients with pituitary tumors showed a decreased magnitude of response in terms of both the rise of 17-hydroxycorticoids as well as duration of this rise, suggesting an impaired capacity to secrete ACTH in response to decreased hydrocortisone secretion by the adrenal. In contrast, patients with adrenocortical hyperfunction of various types showed a very rapid and excessive response in the above mentioned parameters.

Mineralocorticoid-Glucocorticoid Antagonism with Reference to Sodium Excretion

By *Jerry M. Koplowitz, Charles R. Kleeman, Morton H. Maxwell and J. Thomas Dowling.* Wadsworth Hospital, V. A. Center, Los Angeles and Department of Medicine, University of California Medical Center.

The effects on sodium excretion of adrenal corticoids and analogues are well known. However, the sites of action and interaction are still unclear. To evaluate this problem the acute and chronic effects on salt excretion of 2-methyl-9-alpha fluoro-hydrocortisone (2m9αFF, the most potent mineralocorticoid known) with and without the simultaneous administration of Medrol, 6-methylprednisolone (6-MP) were determined.

Three subjects on 100 mEq. sodium diets received 0.25 mg. 2m9αFF twice daily for 3–5 days. Following a recovery phase, this was repeated with 40 mg. 6-MP added daily. Body weight, serum and urinary electrolytes, creatinine, urea and osmolality were measured.

One subject was studied during moderate water diuresis. After equilibration, a single intravenous injection of 0.2 mg. 2m9αFF was administered. The repeat experiment was with 40 mg. 6-MP added. Measurements as above and inulin and PAH levels were determined.

In the chronic studies, 2m9αFF reduced sodium excretion in 2 subjects to less than 6 mEq./24 hrs. throughout the period administered. The 6-MP caused a marked blunting of the sodium retention with respect to both intensity and duration in 2 subjects.

Acutely, 2m9αFF produced a distinct decrease in sodium excretion commencing in 1 hr. There was no change in renal hemodynamics. The repeat experiment adding 6-MP prevented the early anti-natriuresis. In the second situation 6-MP increased GFR and the filtered load of sodium.

The chronic studies indicated that the urine may be made essentially sodium free by the action of a potent mineralocorticoid alone, and that this can be inhibited by the simultaneous presence of 6-MP, a "pure" glucocorticoid. The acute study suggests that the inhibition of sodium retention by 6-MP was partly due to an increase in the filtered load of sodium. A direct antagonism at the tubular level has not been excluded.

Utilization of Oxygen by the Isolated Perfused Rabbit Liver in the Presence of Adrenocortical Hormones

By *Nicholas V. Carroll, Lloyd E. Clayton and Irving Gray.* Research and Development Service, Letterman Army Hospital, San Francisco, and Walter Reed Army Institute for Research, Washington, D. C.

This study was conducted for the purpose of determining the rate of utilization of oxygen by perfused liver tissue and whether this rate is altered in the presence of adrenocortical hormones. Livers were surgically removed from New Zealand rabbits and perfused over a 5-hour period with a 60% whole rabbit blood, 40% saline perfusate. A closed gas circuit apparatus permitted a quantitative measurement of oxygen utilized and carbon dioxide produced by the liver. Hydrocortisone, corticosterone and cortisone were used in the study. The weighed crystalline hormone was made up in a concentration of 700 μg./L. in 6% ethanol and infused at 3 ml./hr. over the 5-hour period. A 6% ethanol solution was infused at the same rate in all control experiments.

Results showed that when hydrocortisone, corticosterone or cortisone were infused, there was a decided increase in oxygen utilization by the perfused liver. When oxygen utilization and carbon dioxide production are increased under the influence of these hormones, the Respiratory Quotient remains essentially the same as that obtained in livers perfused in the absence of cortical hormones. These observations are not in agreement with the results reported in liver slice technics. It has been reported that the cortical hormones have no effect on the uptake of oxygen by liver slices.

Results obtained in this study indicate that

there is an increase in metabolic rate, as evidenced by an increase in oxygen consumption, when certain adrenocortical steroids are available to the perfused rabbit liver.

The Mode of Action of the Spirolactone Compound SC 8109 on Electrolyte Excretion in Normal Human Subjects

By *Roy A. Wiggins, Maxine E. Hutchin, John C. Carbone and Paul D. Doolan.* Clinical Investigation Center, U. S. Naval Hospital, Oakland, California. Supported in part by University of California (San Francisco)/Office of Naval Research Contract Nonr-222(51). The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or necessarily as reflecting the views of the Medical Department of the Navy or the Naval Service at large.

Recently certain synthetic steroids called spirolactones have been shown to induce sodium diuresis and potassium retention in man and animals exposed to the effects of mineralocorticoids. Preliminary data have implicated an antagonism of the aldosterone effect at the level of the renal tubule as the mode of action. The purpose of the present study was to examine this hypothesis in normal young men under the influence of endogenous aldosterone before and during treatment with one of these spirolactones.

Five healthy volunteers were selected. They were working as corpsmen during the test period, and were given a low sodium diet under the supervision of a dietitian to insure constancy. The subjects underwent clearance experiments while on no treatment and while taking 50 mg. of SC 8109 (Searle) intramuscularly every 3 hours. The clearances were performed by the standard technics described for the measurement of the clearance of inulin and sodium para-aminohyppurate, free water clearance and excretion of sodium, potassium, chloride and titratable acidity. The control values were obtained first in 3 patients and after the test period in the remainder.

The administration of a spirolactone was accompanied by a significant increase in sodium excretion, a less striking increase in chloride excretion, a decrease in the potassium excretion and a decrease in titratable acidity excretion. These changes were not attended by any increase in the effective renal plasma flow or by increased glomerular filtration rate.

The data obtained in these studies indicate that the action of spirolactones is confined to the renal tubule. The nature of the observed changes is consistent with a withdrawal of aldosterone effects.

Zoxazolamine as a Uricosuric Agent

By *Elizabeth B. Reed, Thomas V. Feichtmeir and Forrest M. Willett.* V. A. Hospital, San Francisco.

Zoxazolamine, a muscle relaxant, was found to be a potent uricosuric agent, apparently acting by inhibition of renal tubular reabsorption of uric acid. In a series of patients followed for several months to one year, this drug appears to be effective in the treatment of chronic gout.

Zoxazolamine was given orally to normal individuals in single doses of 25 mg. to 500 mg. to determine immediate effects, and in divided doses of 250 mg. to 750 mg. per day to gouty patients as maintenance therapy. Serial determinations of uric acid and endogenous creatinine clearance, prothrombin, bilirubin, WBC, Hgb. and platelet counts were done, in addition to clinical evaluation.

A single dose of 50 mg. was followed by an increase in uric acid clearance within an hour after ingestion which persisted for 5 to 6 hours; the ratio ^{c}urate/^{c}creatinine over the 6-hour period increased from 1½ to 5 times the control value, with considerable variation among individuals. Doses of 100 mg. up to 500 mg. of zoxazolamine, or 250 mg. of probenecid, produced no greater uricosuria than that obtained with 50 mg. in any given individual; however, 25 mg. zoxazolamine usually resulted in a submaximal response.

Of 11 patients with chronic gout treated with zoxazolamine, all responded with increased uric acid clearance, decrease in serum uric acid and no significant changes in other parameters measured. No major side effects were seen.

It is concluded that zoxazolamine is an effective uricosuric agent which deserves further study in the treatment of chronic gout.

Paper Electrophoresis of Porphyrins in Hepatic Cutaneous Porphyria

By *Allan G. Redeker and Rex E. Sterling.* Departments of Medicine and Biochemistry, Uni-

versity of Southern California School of Medicine, and Los Angeles County Hospital.

The porphyrin excretion of patients with hepatic porphyria was studied by means of paper electrophoresis. Extracts of the free porphyrins were electrophoresed at 5 C. using an EDTA: barbital buffer system.

A porphyrin was found which specifically characterized the urinary porphyrin excretion of the hepatic cutaneous porphyrias. This material was completely soluble in aqueous sodium acetate and had absorption characteristics like a uroporphyrin. During electrophoresis this fraction was clearly separated, running just proximal to the fast-moving uroporphyrin. However, its migration was different than would be expected from a 7-carboxyl porphyrin. Separation of this porphyrin from uroporphyrin by adsorbent column chromatography (Al_2O_3, $CaCO_3$, $MgCO_3$) could not be accomplished.

In 18 cases of hepatic cutaneous porphyria, this porphyrin fraction represented from 18% to 53% of the uro-type porphyrin in the urine (average 33%) and was constantly present regardless of the magnitude of the total porphyrin excretion or clinical activity of the disease. As much as 4 mg./day was found in the urine. This porphyrin was not found in the urine in 33 cases of acute porphyria studied, except for trace amounts in a few instances.

In one patient with cutaneous porphyria, fecal porphyrin studies repeatedly showed the fecal content of this porphyrin to exceed by 2-fold the fecal uroporphyrin concentration, a relationship inverse to that found in the urine.

The abnormal porphyrin found in this study is similar to the "pseudouroporphyrin" fraction obtained in in vitro experiments with chicken red-cell preparations. It is apparently type specific for the hepatic cutaneous porphyrias and may be a more important manifestation of the fundamental metabolic defect in these diseases than the increased fecal excretion of proto- and coproporphyrin.

Clinical Evaluation of the "Anhydrous" Fat Emulsion

By *Edward H. Storer.* Department of Surgery, University of Tennessee College of Medicine, Memphis.

Because fat when metabolized yields 9 cals./Gm. as opposed to 4 cals./Gm. from protein or carbohydrate, there is considerable interest in perfecting an i.v. fat emulsion for human use. Such an emulsion would permit a high caloric intake parenterally without excess water loading.

Among the problems encountered have been physical and chemical instability of the emulsion to autoclaving and to prolonged storage at room temperature. Since hydrolysis of the components may be responsible for these difficulties, an anhydrous concentrate has been devised which contains only oil, glycerol and phosphatides. This concentrate is diluted to final volume with 5% D. in W. at the time of administration. These emulsions have proven physically, chemically and biologically stable for at least one year.

About 1200 infusions of this material have been given to hospital patients. Early formulae had to be modified because of high reaction rates, or such undesirable reactions as gross hemoglobinuria. The formula now being used contains in final dilution: safflower oil, 10%; glycerol, 8%; and phosphatides, 0.5%, and yields 710 cals./500 ml. unit.

Four hundred seventy-four infusions of the latest formulae have been given to 251 patients with an infusion reaction rate of 6% and a patient reaction rate of 11%. Multiple infusions have been given to 8 patients with 1 patient receiving 58–500 ml. units over a 2-month period. Some alterations in the coagulogram occur. The earliest and most extensively altered laboratory test has been the BSP retention. This test would appear to afford a simple means of predicting when the "overloading syndrome" is imminent.

Relationship between Phosphate and Cholesterol in the Serum

By *Paul P. Carbone and Kathleen E. Roberts.* U.S.P.H.S. Hospital, San Francisco.

During the course of studies on the effects of phosphate in enhancing the action of thyroid hormone in dogs, it was noted that the serum cholesterol decreased when serum phosphate was increased. Because of the current interest in the relationship of cholesterol to coronary artery disease it seemed advisable to carry out more definitive studies on the relationship between phosphate and cholesterol on serum.

The studies reported here were done on patients and dogs. Experiments were carried out on 14 dogs infused with neutral sodium phosphate at rates sufficient to elevate serum levels

of phosphate. Cholesterol was measured during a control period and following phosphate infusion. In all experiments cholesterol decreased following phosphate infusion. The decrease averaged 67% of the control value and correlated with the degree of phosphate elevation in the serum.

In 5 patients with renal shutdown who were studied, the serum cholesterol decreased coincident to the elevation of serum phosphate which occurs in this situation. The maximum change in cholesterol and phosphate occurred between 9–14 days from the onset of renal shutdown. The decrease of cholesterol averaged 60% of the total and 66% of the free moiety. In 4 of these patients, following dialysis with an artificial kidney, there was abrupt decrease in serum phosphate and an increase in cholesterol to values approaching normal. This increase in cholesterol occurred during the 4–5 hour period of the dialysis. No clear-cut relationship between cholesterol and other serum electrolytes could be demonstrated.

The Response of Blood Lipid Fractions to (ACTH) Stress

By *Richard E. Anderson, Ernest Gold and Paul Starr.* Los Angeles County Hospital, Los Angeles, and Department of Medicine, University of Southern California School of Medicine.

An attempt was made to determine possible effects of "ACTH Stress" on blood lipids in normal individuals on diets varying in fat content.

A group of 5 males and 6 females had their normal diets evaluated for total calories and % fat. Isocaloric low (less than 5%) and high (more than 80%) fat diets were planned from available foods. There were 4 dietary periods: 2 normal diet periods of 2 or 3 weeks' duration, separated by 1 week of low fat diet and followed by 1 week of high fat diet. At the conclusion of each dietary period fasting blood was obtained for cholesterol, lipid phosphorous and alpha and beta lipoproteins. Forty units of zinc corticotrophin were then given intramuscularly and fasting blood obtained 4 hours later for similar lipid studies. Control and post-ACTH 24-hour urines were obtained for total 17-ketosteroids and 17-ketogenic steroids to determine adequacy and possible alteration of adrenal response.

With the altered fat intake, the serum cholesterol and lipid phosphorous showed the anticipated slight fall on low fat intake and slight rise on high fat intake. The change in lipoproteins was inconstant. The pre-ACTH urinary steroids showed little change on low fat intake. There was a 25% average decrease on high fat intake.

Following ACTH there were no significant constant changes in any of the serum lipids on the control or altered fat diets. The post-ACTH urinary steroids increased on all diets. There was no constant modification of response with altered fat intake.

In Vivo Metabolism of Tritiated Thymidine in Man

By *Joseph R. Rubini, Eugene P. Cronkite, Victor P. Bond and T. M. Fliedner.* Medical Research Center, Brookhaven National Laboratory, Upton, New York.

Tritiated thymidine (H^3T) was administered intravenously to 2 patients with brain tumors in whom hematopoiesis appeared normal. Plasma and urine were then serially sampled for nonvolatile tritium activity (NVTA) and tritiated water (THO), and counted in a liquid scintillation spectrometer. Plasma disappearance of NVTA was exponential with 2 components having biologic half times of approximately 2 minutes and 25 minutes. Autoradiographs of bone marrow aspirates revealed minimal nuclear labeling of immature cells as early as one minute. Labelling was heavy in all differentiated precursor cells by 60 minutes, and thereafter, the label was diluted by successive mitoses. Within 2 hours after injection, 20 to 40% of H^3T had been catabolized to THO. Significant amounts of urinary NVTA were detected during the first day; subsequently only very small amounts were excreted. Tritiated beta amino-isobutyric acid (H^3 BAIBA), a catabolic product of H^3 thymine, has been identified in these early urines. THO turned over with a biologic half life of 7–12 days. In one of the patients who subsequently became cachectic and febrile and then received repeated injections, greater amounts of H^3T were degraded to THO and NVTA. The data suggest a dual pathway for H^3T metabolism, i.e., either incorporation into tritiated DNA or prompt catabolism via H^3 BAIBA and THO. Patients with leukemia and other related disorders have been studied similarly, and quantitative differences in H^3T metabolism have been observed.

Effect of Prolonged ACTH or Cortisone Therapy for Rheumatic Fever on 4-Iodoantipyrine Space

By *Jerry K. Aikawa.* Department of Medicine, University of Colorado School of Medicine, Denver.

4-Iodoantipyrine labeled with I^{131} was used as an index of body water to study the change in body composition induced by prolonged ACTH or cortisone therapy of active rheumatic fever. Fifty μc. of I^{131} was injected intravenously and blood obtained at 2, 4 and 6 hours. The concentration found by extrapolation to zero time was used to calculate the antipyrine space (AP). Body fat (F) was calculated from the formula, 100−(% water/0.732). Exchangeable potassium content (Ke) was determined by the K^{42} isotope dilution technic.

A boy of 12 with upper respiratory infection without rheumatic fever had a Ke value of 46.8 mEq./Kg., AP of 55.6% of body weight and F of 24.0%; these values agree with those found previously in normal adolescents. A boy of 9 on the 2nd day of cortisone therapy for acute rheumatic fever had a Ke of 43.5 mEq./Kg., AP of 46.7% and F of 36.2%. A girl of 8 on cortisone, 150 mg. daily, for 44 days had a Ke of 41.8 mEq./Kg., AP of 44.6% and F of 39.1%. A boy of 10 treated with ACTH, 100 mg. maximum daily, for 154 days had the following: Ke of 34.0 mEq./Kg., AP of 38.3% and F of 47.7%. Maximum aberrations were found in a girl of 14 given ACTH, up to 100 mg. daily, for 119 days: Ke of 34.3 mEq./Kg., AP of 31.9%. and F of 56.4%. All 4 rheumatic subjects gained weight.

A progressive decrease in AP occurred as Ke/wt. decreased and body weight increased. The results are interpreted as indicating that body fat increased as muscle mass and water content decreased. These findings confirm the clinical impression that these children were becoming progressively fatter.

Normal Serum Electrolyte Values in Term and Premature Infants

By *Robert S. Cox, Jr., James K. Akiyama, Walter L. Fitch, Sheldon F. Freedman, Richard W. Whitney and Vincent J. Pileggi.* Research and Development Service, Letterman Army Hospital, San Francisco.

Plasma electrolyte values were determined on a series of 50 newborn term infants on the 1st, 3rd and 5th days of life. In addition, 11 healthy premature infants were studied approximately every other day until discharged from the hospital, some 18 to 60 days after birth. Seven sick premature infants were similarly studied until demise or cure.

Determinations of CO_2 content, hematocrit, chloride, total protein, sodium, potassium and glucose were made by ultra microchemical methods modified from those of Natelson. The methods were studied for precision and accuracy.

The methods were all found to be highly satisfactory and precise. The outstanding findings were an extremely variable blood glucose with extremely low values noted in apparently normal infants and a tendency especially in premature infants toward extremely high plasma potassium levels, indicative of their moderate hypoadrenalism. The standard constituents studied are summarized, giving a firm basis for blood levels of these constituents in the newborn and premature period.

Histamine Metabolism in Human Disease

By *Gildon N. Beall and Paul P. VanArsdel, Jr.* Department of Medicine, University of Washington School of Medicine, Seattle.

When C^{14} histamine is given intravenously to humans, it is rapidly cleared from the plasma, and all administered radioactivity soon appears in the urine. The purpose of this study was to establish the nature of the urinary metabolites in 6 normal humans, 3 asthmatics, 3 patients with histaminic cephalalgia, 5 cirrhotics, and 1 patient with uremia, following administration of 100 μg. of C^{14} histamine.

Two-dimensional ascending paper chromatography of a methanol extract of the subjects' urine consistently revealed 3 radioactive metabolites detectable by radioautography. One was thought to be imidazoleacetic acid-riboside; an average of 23% of the elutable radioactivity was recovered from this area. The 2nd was imidazoleacetic acid (ImAA); this contained over 50% of the radioactivity. The 3rd was probably 1,4-methylimidazole acetic acid; 23% of the radioactivity could be eluted from this area. Occasionally, traces of a 4th metabolite were noted. This was apparently methyl-histamine.

There were no abnormalities in the nature of the urinary metabolites in any of the condi-

tions studied, with the exception of the patient with uremia whose chromatogram showed only ImAA riboside. ImAA was the principal metabolite in most instances, but the relative amounts of each metabolite varied considerably. A patient with histaminic cephalalgia was "desensitized" to histamine. No change in histamine metabolism could be detected following this procedure. From these studies, it can be concluded that histamine inactivation and degradation in man is a rapid and complete process which is not readily altered in disease.

GASTROINTESTINAL SYSTEM

Experimental Production of Atrophic Gastritis

By *Edward H. Storer and Francis C. Nance.* Department of Surgery, University of Tennessee College of Medicine, Memphis.

Atrophy of the gastric mucosa is relatively common in the human and is of considerable importance because of its probable role as a precursor of gastric carcinoma. Gastric atrophy rarely if ever occurs spontaneously in animals. A reproducible method for producing mucosal lesions closely resembling atrophic gastritis, both functionally and histologically, has been used successfully in the dog.

Heidenhain pouches were made, and baseline secretion studies and control biopsies performed. The mucosa of the pouch was then destroyed by burning the surface for brief periods with hot water. Most animals required several treatments to attain an atrophic mucosa.

Significant data have been obtained on 24 animals. Histamine-fast achlorhydria lasting up to 9 months after trauma was produced. All animals resumed acid secretions after variable periods, but at a level less than 25% of control values. Achlorhydria can be maintained by reapplication of thermal trauma.

Histologically, the mucosa demonstrates irregular, shallow glands, sparse or absent parietal cells and occasional small, round cell infiltrations and cyst formation.

The method is simple, reproducible, can be reapplied as needed to maintain an atrophic mucosa, and the animals maintain a good nutritional state.

Gastric Ulceration in Experimentally-induced Polycythemia

By *James R. Dahl, Richard K. Blaisdell and Ernest Beutler.* Argonne Cancer Research Hospital, USAEC, and Department of Medicine, University of Chicago.

The increased incidence of peptic ulcer in patients with polycythemia rubra vera has never been explained satisfactorily. As a first step in an investigation of this problem, an attempt was made to demonstrate a similar relationship between polycythemia and ulceration in the experimental animal.

Rats were rendered polycythemic by the administration of washed and packed homologous rat erythrocytes via tail vein infusion. The erythrocytes were administered in 3 doses, given at 2-day intervals, to a total dosage of 8% of the body weight. In 2 control groups, normal rat plasma and isotonic saline, in equal amounts and administered in an identical fashion, were substituted for the packed erythrocytes. By the 6th day, hematocrit levels greater than 75% were observed in all the animals that received erythrocytes, whereas the hematocrit levels of the other groups showed no consistent change from preinjection values.

All animals were autopsied 3 days following the final infusion. Apart from generalized hyperemia, abnormalities in the polycythemic animals were confined to the stomach. The stomachs of 10 of the 13 animals showed ulceration of a type that resembled human peptic ulcers both grossly and microscopically. The stomachs of the 3 remaining rats of this group were also abnormal, demonstrating multiple small areas of superficial hemorrhagic gastritis. No lesions were present in any of the animals of either of the control groups.

It has been demonstrated, therefore, that gastric ulceration in the rat can be produced by transfusion-induced polycythemia. These findings suggest an interesting parallel between this experimental system and the patient with polycythemia rubra vera and its associated increased incidence of peptic ulcer. Additional studies are in progress to determine whether gastric hypersecretion of acid occurs secondarily to the induced polycythemia, or whether, in its absence, vascular factors alone may initiate ulcer formation.

The Excretion of Indocyanine Green and its Use in the Estimation of Hepatic Blood Flow

By *Sigmund G. Ketterer, Bernard D. Wiegand and Elliot Rapaport.* Cardiopulmonary Laboratory, Mount Zion Hospital, San Francisco.

The rapid intravascular disappearance of the protein-bound dye, indocyanine green, after its intravenous use in registering indicator dilution curves suggests that it is excreted primarily by the liver. The purpose of the present investigation was to study the excretion of this dye in the normal dog with the view toward its possible use in the estimation of hepatic blood flow.

It was found that following cessation of a constant infusion, indocyanine green leaves the circulation rapidly in a non-exponential fashion with approximately a 50% decrease in concentration after 15 minutes and 80% after 1 hour. Significant arteriovenous differences in concentration were not demonstrated across the extremities, kidneys and lungs during constant infusion of the dye, nor was the dye detected in urine or spinal fluid. Simultaneous sampling from the femoral artery and hepatic vein demonstrated an arterial hepatic venous difference in dye concentration which was obliterated following ligation of the bile ducts. Extraction ratios average 18% (range 11 to 24%). Recovery of the dye in bile collected from surgically created biliary fistula dogs has yielded up to 89% and averaged 77% of the injected dye. Estimation of hepatic blood flow by the constant infusion method of Bradley, substituting indocyanine green for bromsulphalein, average 38.7 cc./min./Kg. This is in good agreement with previously reported results using bromsulphalein.

It is concluded that indocyanine green is excreted primarily by the liver, and that extrahepatic removal is sufficiently small not to preclude its use in the estimation of hepatic blood flow in the dog.

Measurement of Hepatic Blood Flow in Dogs by the Indicator Dilution Technic using Indocyanine Green

By *John F. Murray, John Rafferty and Robert Maddox.* Department of Medicine, University of California Medical Center, Los Angeles.

A new dye, indocyanine green, was used to measure hepatic blood flow (HBF) by the indicator dilution technic. At laparotomy a 0.038" (O.D.) polyethylene catheter for dye injection was passed via a distal mesenteric vein into the portal vein. This procedure required a minimum of surgical intervention, and ligation of a small mesenteric vein presumably did not alter total HBF. Blood was withdrawn from a hepatic vein catheter through a cuvette-densitometer giving a continuous time-concentration curve.

Thirty determinations of HBF were performed in 9 dogs. When small amounts of dye were injected, erroneously high flows resulted from a small uptake of dye during the initial passage through the liver. Extrapolation of a dose-flow curve indicated that dye extraction was approximately 0.015 mg./Kg. After correcting for uptake using this figure the mean HBF was 46.2 (S.D. 10.7) ml./Kg./min. In 2 dogs, sampling from different hepatic veins gave similar flows.

In 4 dogs, large amounts of dye were successively injected into the portal and femoral veins and sampled from the inferior vena cava above the diaphragm. Similar flows resulted, showing that with large amounts of dye the % uptake by the liver becomes small relative to the total dose and that no correction factor is needed. The mean uncorrected HBF in dogs receiving greater than 0.070 mg./Kg. of dye was 48.6 (S.D. 8.8) ml./Kg./min.

Total flows calculated by the dye-dilution method are slightly higher than results obtained by clearance technics; this suggests that a small fraction of total HBF perfuses areas without functioning parenchymal cells.

An Effective Form of Therapy of Refractory Ascites in Cirrhosis of the Liver

By *Allan G. Redeker, Oliver Kuzma and Telfer B. Reynolds.* Department of Medicine, University of Southern California School of Medicine, and John Wesley County Hospital, Los Angeles.

Ascites complicating cirrhosis of the liver is often refractory to any diuretic regimen. Organomercurials alone are frequently ineffective, and their potentiation with ammonium chloride is hazardous because of possible ammonium toxicity. Chlorothiazide alone often fails to induce natriuresis.

In this study, a combination of 6-methylprednisolone and chlorothiazide was found to be consistently effective in producing a sodium diuresis in 5 patients with refractory ascites. These patients were hospitalized, maintained on a constant low sodium intake and followed with daily

body weights and frequent 24-hour urine sodium determinations. Failure of sodium diuresis with chlorothiazide and mercurial therapy was the basis of patient selection for this study. Urinary sodium excretion in the 5 patients during chlorothiazide administration (2 Gm./day) ranged from 0 to 19 (mean 8) mEq./24 hours. When 6-methylprednisolone alone (12 mg./day) was administered, urine sodium increased only slightly, ranging from 0 to 28 (mean 13) mEq./day, and there was no loss of weight. The concurrent administration of chlorothiazide (2 Gm./day) and 6-methylprednisolone (12 mg./day) resulted in sustained natriuresis and loss of ascites, with urinary sodium ranging from 15 to 122 (mean 53) mEq./day. The average weight loss during therapy was 23 pounds. If, during this combined therapy, either chlorothiazide or the steroid was discontinued, urinary sodium promptly decreased to pretreatment levels and loss of ascites ceased. No significant change in the levels of the plasma proteins was observed.

The possibility of alterations in aldosterone metabolism influencing the sodium diuresis provoked by combined 6-methylprednisolone and chlorothiazide therapy is being investigated.

GENITAL TRACT

Penetration into the Human Ovarian Follicular Fluid of Tracers and Drugs

By *Kurt N. von Kaulla, Jerry K. Aikawa and John D. Pettigrew.* Departments of Medicine and Obstetrics and Gynecology, University of Colorado School of Medicine, Denver.

This is an exploratory study concerning the transfer into human ovarian follicular fluid of various substances administered orally or parenterally. Twenty-nine women in the child-bearing age were given the following materials at various time intervals prior to hysterectomy by the routes indicated (n = number of observations) : I^{131} (i.v., n = 14), I^{131}-human gamma globulin (i.v., n = 6), Hg^{203}-mercaptomerin (i.v., n = 3), C^{14}-meprobamate (i.v., n = 4), penicillin (i.m., n = 4) and Pyridium (p.o., n = 6). The ovaries were exposed during surgery and follicular fluid aspirated from a prominent follicle. A venous blood specimen was obtained simultaneously. Sixteen histologic preparations of the punctured follicles were obtained; 6 were fresh corpora lutea. I^{131} and Hg^{203} were assayed with a well scintillation counter; C^{14} with a windowless flow counter; penicillin by assay of its bacteriocidal activity; and a qualitative test was employed for Pyridium. The concentration of the various substances in the follicular fluid was expressed as the ratio, follicular fluid/serum.

The substance administered, the time interval of administration prior to surgery, the range of ratios and the mean ratio were, respectively: I^{131}—¼ to 7 hrs., 0.14–0.97, mean of 0.44; I^{131}-gamma globulin—13 to 21 hrs., 0.09–0.81, mean of 0.45; Hg^{203}-mercaptomerin—11 to 12 hrs., 4.20–19.70, mean of 14.18; C^{14}-meprobamate—2½ to 11 hrs., 0.59–1.40, mean of 1.03; penicillin—2 to 4½ hrs., 0–0.50, mean of 0.33. Pyridium was detected qualitatively in the follicular fluids.

The data show that various compounds administered orally or parenterally not only penetrate rapidly into the human ovarian follicular fluid but that they may be concentrated therein. This property might be particularly meaningful when exhibited by a beta-emitting radioisotope or by drugs with mutagenic or radiomimetic effects. We are at present attempting to determine some of the effects of the presence of such material within the follicular fluid on the ovum.

Fluorescence Method for Exfoliative Cytology Screening

By *Robert S. Cox, Jr. and John F. Pelan.* Research and Development Service, Letterman Army Hospital, San Francisco.

An evaluation study was undertaken to determine the effectiveness of fluorescence microscopy in detecting malignant cells in vaginal and cervical smears, as compared with the accepted Papanicolaou stain method. The smears were obtained in the usual manner and stained with acridine orange. The wet preparations were then examined with a fluorescence microscope assembly utilizing a mercury arc lamp with a substage blue violet exciter filter and a yellow barrier in the eyepiece.

A total of 1025 cases were studied, with 85 determined to have atypical characteristics by either the fluorescence or Papanicolaou method.

One case proven to have carcinoma of the cervix was called normal by fluorescence and atypical by Papanicolaou technic. No other positives were missed by the fluorescence method, and although a number of "false" atypicals were found, the amount of time saved by this method warrants its adoption. A slide can be screened in approximately 5 minutes instead of 20 minutes and once screened by the fluorescence technic can be restained and examined with the Papanicolaou stain if indicated.

The fluorescence method has indicated a high degree of reliability in our hands with a marked saving of time.

INFECTIOUS DISEASE

Tellurite Reduction as an Indicator of Staphylococcal Virulence

By *Paul D. Hoeprich, Garth F. Croft and Lionel M. West, II.* Departments of Internal Medicine and Pathology, University of Utah College of Medicine, and Clinical Microbiology Laboratory, Salt Lake County General Hospital, Salt Lake City.

Coagulase production by *Staphylococcus* species has been found to correlate better with virulence than production of pigment, hemolysin(s), kinase or protease. However, the tube coagulase test is both laborious and time consuming, requiring an overnight culture for inoculation and serial observations over an 18-hour period once the test is set up.

Since tellurite reduction was found to be accomplished by a few species of *Staphylococcus* which were coagulase producers, Zebowitz et al. recommended a tellurite-glycine agar medium for selective isolation of pathogenic *Staphylococcus* species.

Two hundred and fifty-one isolates of *Staphylococcus* from clinical materials were studied using: (1) tube coagulase test—readings at ½, 1, 2, 6, 18 and 24 hours after inoculation; (2) 20% plasma agar—incubated 48 hours at 30 C.; (3) sheep blood agar—incubated in a candle jar for 18 hours at 37 C.; (4) Chapman Stone agar—incubated 48 hours at 37 C.; (5) Tellurite-glycine agar—incubated for 24 hours at 37 C.

Reaction on plasma agar, blood agar and the Chapman Stone medium was in poor agreement with test tube coagulation of plasma. On the other hand, there was good agreement between tellurite reduction (193 positive out of 251 tested) and tube coagulation of plasma (191 positive out of 251 tested): (1) 5 of the 251 isolates were coagulase positive, but tellurite negative; (2) 7 of the 251 isolates were tellurite positive, but coagulase negative; (3) 239 of the 251 isolates displayed consistent reaction with plasma and tellurite—186 both coagulated plasma and reduced tellurite; 53 neither coagulated plasma nor reduced tellurite. Ability to coagulate plasma and reduce tellurite correlated well with clinical evidence for pathogenicity.

Ease of use and reliability recommend determination of ability to reduce tellurite, instead of coagulase assay, as a laboratory criterion of pathogenicity of isolates of *Staphylococcus* species.

Long-term Follow-up on Ototoxicity and Nephrotoxicity of Kanamycin

By *Sydney M. Finegold and Edward A. Kantor.* Departments of Medicine and Surgery, Wadsworth Veterans Hospital, and University of California Medical Center, Los Angeles.

This study was designed to determine the incidence and extent of the nephrotoxic and ototoxic effects of kanamycin and to note any changes after discontinuation of therapy. Over 100 patients receiving kanamycin parenterally for a variety of infections were studied serially with urinanalyses, serum creatinines, audiograms and caloric tests. A few have follow-up of 6 months after discontinuation of treatment.

There was a distinct correlation between dosage and duration of therapy and ototoxicity and between age and nephrotoxicity. There was a very striking relationship between impairment of renal function (kanamycin-induced or otherwise) and ototoxicity.

Minor changes in the urinary sediment were seen commonly but were of no significance and usually disappeared within one week of discontinuation of treatment. Significant elevation of serum creatinine due to kanamycin therapy was seen in 12 patients; this was important only as it predisposed to VIII nerve damage. The creatinine usually returned to normal 3 or 4 weeks

after treatment was stopped, but in 2 instances it took 2 months or longer.

Ototoxicity was usually bilateral, and the auditory portion of the VIII nerve was involved much more often than the vestibular. There was a distinct tendency to involve high frequencies, so that many patients with audiographic evidence of VIII nerve toxicity did not suffer conversational hearing loss. Tinnitus was often, but not always, an early warning of VIII nerve damage. Following discontinuation of the drug, equal numbers of patients showed improvement or worsening of the audiogram; changes always occurred within a week of discontinuing the drug.

Renal damage after kanamycin therapy is mild and reversible, though often slow. VIII nerve damage is at times reversible and is not progressive over long periods after discontinuance of drug therapy as has been noted with other drugs.

A Medical and Psychosocial Study of Tuberculous Patients Who Relapse to Infectious Sputum during Hospitalization

By *James E. Hart, Joan K. Jackson and Thomas H. Holmes.* Department of Psychiatry, University of Washington School of Medicine, and Firland Sanatorium, Seattle.

This research evaluates the importance of 3 factors contributing to relapse in sputum status. These are: (1) change in disease status (caseation, spread or bronchial communication), (2) drug therapy and complications (side reactions or resistant organisms), and (3) psychosomatic factors (social and emotional problems leading to physiologic and behavioral changes).

Patients studied had positive sputum (containing tubercle bacilli) on admission, converted to negative sputum, maintained negative sputum for at least 3 consecutive months of hospitalization and then demonstrated positive sputum (relapse) again. Each patient was assigned a control matched as to extent, type and localization of disease, age and sex, but who remained negative or free from tubercle bacilli.

Results: Patients had more side reactions to drugs, but had equal numbers and dosages of the anti-tuberculosis drugs for equal time periods. More controls had organisms resistant to drugs. More controls had unstable, changing disease. On current x-rays and changes or complications in disease, more controls were considered by the experts to have a high probability of relapsing to infectious sputum.

Patients and controls differed greatly in social background and life accomplishments. Patients' histories were marked by a consistent pattern of failure in adjusting to life situations and in maintaining interpersonal relationships.

Patients had greater difficulties in adjusting to the hospital and to their roles as tuberculous patients. Confronted with similar types and numbers of social and emotional problems during hospitalization, patients and controls reacted differently. Controls could utilize emotional and social resources to resolve problems. Patients had greater difficulties in handling these problems with resultant emotional upheaval. These periods of crisis exactly dove-tailed with the time period in which positive sputum developed.

Predictions of relapse in sputum status within one year were made for each member of the control group, and were 100% accurate.

A Comparison of American Trudeau Society Medium and Middlebrook-Dubos "7H9" Medium in the Isolation of *M. tuberculosis*

By *H. W. Harris, Merle J. Selin and Ralph A. Knight.* Medical and Laboratory Services, V. A. Hospital, and Department of Medicine, University of Utah College of Medicine, Salt Lake City.

Two different bacteriologic media, both widely used for the cultural detection of *M. tuberculosis,* have been compared. Each of 6,373 decontaminated, clinical specimens were cultured on 2 tubes of ATS (American Trudeau Society) medium and 2 tubes of "7H9" (Middlebrook-Dubos, Oleic Acid-Albumin-Agar) medium. The media were compared for the isolation of *M. tuberculosis,* the number and size of colonies and the incidence of contamination.

Both media yielded negative cultures in 4,778 specimens. Positive cultures for *M. tuberculosis* were found on both media in 709 specimens. The number of colonies on ATS medium was twice that on 7H9 in 74 specimens; in 6 specimens 7H9 produced twice as much growth as ATS. Colonies on ATS medium appeared slightly larger than those on 7H9. Complete contamination occurred in 885 of 12,746 tubes of ATS medium (7%) and in 326 of 12,746 tubes of 7H9 (2.6%). This difference is not statistically significant.

M. tuberculosis was cultured from 45 specimens only on ATS and from 24 specimens only on 7H9. This difference is not statistically sig-

nificant. Had 7H9 medium not been used and cultures made on only 2 tubes of ATS, 24 specimens would have been falsely reported negative and 137 lost by contamination of both tubes. Had 2 tubes of 7H9 medium only been used, 45 specimens would have been falsely reported negative and 16 specimens would have been lost by contamination. The index of error, e.g., false negatives plus the specimens lost by contamination, totals 161 for ATS and 61 for 7H9. This difference is statistically significant and favors the 7H9 medium. In this routine clinical bacteriology laboratory, ATS is a slightly more sensitive medium than is 7H9, but, due to a lower rate of contamination, 7H9 offers greater practical advantage. Best results are obtained by using both media.

Concerning the Antituberculous Effect of Iproniazid Used as a Neuropsychiatric Drug

By *William Mandel and Seymour M. Farber.* Department of Medicine, University of California School of Medicine, San Francisco.

Iproniazid (IPH) was abandoned as an antituberculous drug when it was found to have a greater incidence of side-reactions than isoniazid (INH). IPH has recently been used in neuropsychiatric subjects because it inhibits monoamine oxidase, a property not possessed by INH. When used for this purpose, its known antituberculous activity has been ignored.

Microbiologic assays for INH were performed on serum of 5 subjects 2, 4 and 6 hours after administration of 300 mg. of IPH and at similar intervals after 300 mg. of INH. The results were lower after IPH than after INH, the difference being statistically significant. Detectable values, however, were present in the serum of all subjects.

The currently recommended daily dose of IPH is 50 mg., though the previously recommended daily dose was 150 mg., and amounts as high as 600 mg. have been given. It is unlikely that 50 mg./day is sufficient to maintain adequate antimicrobial levels. Despite this, if IPH is used in undiagnosed tuberculous subjects, drug-resistant mutants may emerge from what is ineffective single drug treatment. Since tubercle bacilli show cross resistance to IPH and INH in vitro, organisms resistant to IPH will probably be resistant to INH. Wide and injudicious use of IPH may therefore conceivably be harmful to undiagnosed tuberculous subjects.

IPH in low doses might kill small numbers of tubercle bacilli and prevent conversion of the tuberculin skin test in recently exposed subjects. Thus, IPH could fortuitously serve as a chemoprophylactic agent.

The Accuracy of Diagnosis of Influenza Using Clinical Criteria

By *C. R. Dawson and K. Uyeyama.* U.S. Public Health Service, and Student Health Service, University of California Medical Center.

Is it possible to separate influenza from other respiratory diseases on clinical grounds alone? To examine the feasibility of diagnosing influenza clinically, cases of acute respiratory disease occurring during the 1957 outbreak of "Asian" influenza were evaluated according to the diagnostic criteria listed in 6 standard reference sources.

Clinical histories and physical examinations were recorded by 2 physicians. Throat washings for virus isolations and acute and convalescent sera for complement fixation tests were taken from each patient to establish a laboratory diagnosis. The recorded symptoms and clinical findings of each patient were then compared with the above described set of diagnostic criteria. Cases exhibiting 60% of the criteria listed in the reference were considered to be diagnosable.

In 17 of 19 patients influenza virus infection was unequivocally established by virus isolation or antibody titer. The attending physicians claimed the diagnosis of influenza in 15 of the 17 individuals, and also in about half of a comparable group of proven non-influenzal etiology. The standard criteria of clinical influenza, on the other hand, were present in only 9 to 11 of the 17 established cases. Thus it is clear that the attending physicians' diagnosis was heavily prejudiced in favor of the diagnosis "influenza" by the existence of a known influenza epidemic and included many non-influenza cases, whereas about half of the actual influenzal infections were quite atypical and could not be diagnosed by standard clinical criteria.

KIDNEY

The Relationship between Urinary Specific Gravity and Urinary Osmolarity in Normal Adults

By *Edgar J. Schoen.* Department of Pediatrics, Kaiser Foundation Hospital, Oakland, California.

The reliability of urinary specific gravity readings as a measure of urinary total solute concentration over the parameters of urinary dilution and concentration was studied in 333 urine specimens collected from 12 normal adults having no evidence of glycosuria or proteinuria. Urinary specific gravity in the high range (above 1.020) reflected well urinary osmolarity and represented osmotically concentrated urine (above 800 mOsm./L.). Urinary specific gravity below 1.005 represented urinary osmolarity below that of serum. In the range between 1.005 and 1.020, the specific gravity reading often proved misleading as an indicator of urinary total solute concentration. Neither the urinary urea concentration nor the urinary urea to salt ratio predicted the relationship between specific gravity and total osmolarity in individual urine specimens.

Direct measurement of urinary osmolarity offers the following advantages over specific gravity measurement: (1) direct comparison of concentrations of total urinary solutes with individual urinary solutes; (2) direct comparison of solute concentrations in urine and other body fluids; (3) greater accuracy; (4) enhanced adaptability to micromethods; and (5) diminished need for prolonged thirsting in tests for renal-concentrating ability.

Studies on the Mechanism of Bicarbonate Reabsorption in Man

By *George B. Gordon, Alfred Eichenholz, Frank MacDonald and Thomas Semba.* Veterans Hospital, Minneapolis, Minnesota, and Department of Medicine, University of Minnesota.

The purpose of this study was to determine what fraction of renal bicarbonate reabsorption depends upon $H^+ - Na^+$ exchange.

Seven males without evidence of renal disease were investigated. Inulin clearance was measured. The serum or plasma was analyzed for CO_2 content, pH, Na^+, K^+, Cl^- and P (inorganic phosphorus). Similar analyses were carried out on anaerobically collected urine. Bicarbonate was calculated from the Henderson-Hasselbalch equation. The filtered loads of the various ions were calculated as the products of the inulin clearances and their serum concentrations. Reabsorption was calculated as the difference between the filtered load and urinary excretion. Following appropriate control periods constant intravenous infusions of acetazolamide in doses of 100 to 120 mg./Kg. body weight were given over an hour's time.

The effects were almost immediate. In all the subjects there was a significant reduction in filtration rate. Urine volume increased dramatically. Urinary pH and pCO_2 exceeded that of the blood. No significant changes occurred in plasma pH, CO_2 content or electrolyte concentrations. Bicarbonate reabsorption expressed in millimoles reabsorbed per 100 cc. of glomerular filtrate decreased by 40%. The reabsorption of Na^+, Cl^- and P expressed as the % of the filtered loads exhibited reductions from 99%, 98% and 91% to 90%, 94% and 73%, respectively. K^+ excretion frequently exceeded filtered load.

Since the reaction, $H_2O + CO_2 \rightleftarrows H_2CO_3$, approaches 90% equilibrium in about 200 seconds at 38 C. in the absence of carbonic anhydrase, the urinary diversion of 40% of filtered bicarbonate by acetazolamide suggests that $H^+ - Na^+$ exchange accounts for the majority of bicarbonate reabsorption in man.

The Effect of Intravenous Administration of Pyrazinamide and Tubular Reabsorption of Uric Acid

By *Fariborz Amini, Nicholas L. Petrakis, William Mandel and Marie Doherty.* Cancer Research Institute, University of California School of Medicine, San Francisco.

Hyperuricemia has been reported with prolonged administration of Pyrazinamide (in tuberculous patients), and it has been shown by Yu et al. that there is decreased renal uric acid clearance after a single oral dose of Pyrazinamide. The purpose of the present study was to determine the effect of intravenous administration of Pyrazinamide on the tubular reabsorption of uric acid.

Uric acid and creatinine clearance determinations were carried out in 7 patients in 9 consecutive hourly periods, 3 hours before and 6 hours after the administration of Pyrazinamide.

Pyrazinamide was administered intravenously as a dose of 25 mg./Kg. in 2% solution in about 2 to 4 minutes.

The urines were collected through an indwelling catheter, and the bladder was washed with normal saline at the end of each collection period. Serum uric acid levels were determined every hour during the study and serum creatinine was determined at the beginning and 3 and 6 hours after the administration of Pyrazinamide.

The present studies demonstrate that the intravenous administration of Pyrazinamide results in a definite increase in tubular reabsorption of uric acid occurring 1 to 2 hours after injection, which reaches a maximum at 3 to 4 hours and then declines. Significant alterations in serum uric acid levels were not observed during the study period.

A Functional and Morphologic Follow-up Study of Acute Renal Failure

By *John D. E. Price and Russell A. Palmer.* Department of Medicine, Vancouver General Hospital, University of British Columbia, and British Columbia Medical Research Institute.

Fourteen patients who have recovered from acute renal failure were studied at varying intervals from a few weeks to 10 years after their acute illness. Clearance studies, concentration test and P.S.P. excretion were done among other functional tests. In 8 patients a renal biopsy was performed in an attempt to correlate the functional with the morphologic findings.

The findings were consistent with previous reports in that renal function approached normal in the majority of cases within 6 months of the acute illness, except in those cases with other diseases affecting the kidneys, such as essential hypertension.

In view of the small number of cases available, it was not found possible to correlate functional and morphologic findings. However the histologic changes in the kidney, which were present in all cases, were thickening of the glomerular, Bowman's capsular and tubular basement membranes in focal areas. There were also focal areas of interstitial scarring and tubular atrophy. In the 2 cases in which biopsy was performed shortly after diuresis commenced, there was marked cellularity of the glomeruli, and this was also noticed to a lesser degree in patients studied at 6 months after their acute illness. Those seen more than 12 months after the initial shutdown did not show hypercellularity. The glomerular changes appeared to be the most striking.

The conclusion drawn was that while renal functional studies confirmed the findings of previous workers, the persisting morphologic changes found in the glomeruli and tubules suggest that full resolution does not take place. The glomerular changes raised the question of whether the primary lesion in acute renal shutdown is in the glomeruli, as originally put forward by previous workers in this field who have suggested the name glomerolonephrosis to describe the pathologic changes.

The Defect in Urinary Dilution Associated with Chronic Renal Disease

By *Charles R. Kleeman, Donald A. Adams and Morton H. Maxwell.* Department of Medicine, V. A. Center, Los Angeles, and Department of Medicine, University of California Medical Center.

The impairment in concentrating ability in chronic renal disease has been critically evaluated; the diluting defect has not. The present study undertook an evaluation of this defect.

If impaired diuresis were due to reduced functional renal mass, and inulin and PAH clearances were reasonable approximations of residual mass, predicted values for urinary flow (V) and free-water clearance ($(Ch_2o)/100$ cc. of glomerular filtrate (gfr) and minimal osmolality (mOsm./L.) could be calculated by extrapolation to normal renal mass (mean normal gfr/1.73M.^{2}). These could be plotted as a function of the predicted rate of solute excretion

$$(\mu Osm./min./1.73M.^2 \times \frac{\text{mean normal gfr}}{\text{observed gfr}})$$

compared to maximal normal water diuresis with increasing solute loads.

Predicted values for V and $Ch_2o/100$ cc. gfr greater than, and mOsm./L. less than, in normal subjects would suggest a defect causing a diminished water reabsorption in the residual nephrons. The converse predicted values would suggest an increased permeability to, or an increased reabsorption of, water.

Twenty-one patients with various chronic renal diseases were studied while in a steady azotemic state. Maximal diuresis was attained by a sustained water load of 1,000 cc. for 5 hours. All values were corrected to 1.73M.^{2}

Mean inulin and PAH clearances, V, Ch_2o and mOsm./L. were 14 cc./min., 55 cc./min., 3.9 cc./min., 1.8 cc./min., and 168 mOsm./L., respectively. Mean predicted rate of solute excretion (μOsm./min./1.73M.^{2} x $\frac{\text{mean normal gfr}}{\text{observed gfr}}$) was 7,000 mOsm./min.

Extrapolation of the above results to 100% renal mass and comparison with normal subjects disclosed the following: 10 patients had predicted values suggesting a decreased water reabsorption in the residual nephrons; 7 had values suggesting "normal" residual nephrons; while in only 4 subjects did the values suggest increased reabsorption of water.

Maximal water diuresis in severe chronic renal disease is usually equal to or greater than normal when calculated on the basis of normal renal mass and predicted solute diuresis.

Homeostatic Responses to Blood Volume Sequestration in Normal Pregnancy

By *W. J. Dignam, N. S. Assali and K. Dasgupta.* University of California Medical Center, Los Angeles.

The homeostatic responses to venous pooling were investigated in 21 normally pregnant subjects, divided into 3 groups. In the 1st group, renal hemodynamics and water and electrolyte excretion were studied during quiet standing and venous occlusion of the lower limbs in the 1st, 2nd and 3rd trimester of pregnancy and post partum. In the 2nd group, the effects of oral and intravenous alcohol on the antidiuresis and antinatriuresis of quiet standing were investigated. In the 3rd group, aldosterone excretion was determined in periods of 12 hours of ambulation alternated with similar periods of recumbency.

Venous pooling in pregnancy, regardless of whether it is induced by quiet standing or venous occlusion, produced a marked fall in glomerular filtration rate, renal plasma flow, urine flow, sodium and chloride excretion and a lesser fall in potassium excretion. Osmolal and free water clearances also fell significantly, the latter becoming negative in the majority of instances. These changes increased steadily with the progress of gestation and reverted to non-pregnant patterns after delivery.

Contrary to its action in non-pregnant subjects, ethyl alcohol did not inhibit the antidiuresis of quiet standing in pregnancy, neither did it affect the other renal changes. Aldosterone excretion increased markedly during the periods of standing and ambulation and the increase coincided with the fall in Na excretion.

The data suggest that in pregnancy, because of the greater tendency to venous pooling due to the heavy uterus and the increased intra-abdominal pressure, sequestration of the effectively circulating blood volume by standing or venous occlusion produces marked renal hemodynamic changes which are the primary factors responsible for the antidiuresis and antinatriuresis. Increased aldosterone excretion may enter into play later and sustain the antinatriuresis even after the return of the renal hemodynamics to normal levels. ADH seems to play a secondary role.

Triamcinolone Therapy of the Idiopathic Nephrotic Syndrome (Lipoid Nephrosis) in Adults

By *Thomas A. Marr, John H. Lindberg and H. Arnold Muller.* Department of Medicine, University of Washington, Seattle.

The effects of triamcinolone therapy in a group of 14 adults, 11 males and 3 females, having uncomplicated nephrotic syndrome have been studied. All patients manifested edema, hypoalbuminemia, hypercholesterolemia, proteinuria in excess of 4 Gm./day and oval fat bodies in the urine sediment. None had hypertension, hematuria, renal insufficiency, anemia or serum electrolyte abnormalities. Their ages ranged from 19 to 78 years. Six were over 55 years of age.

Percutaneous renal biopsy performed in 11 cases has shown changes of membranous glomerulonephritis. The lesion is characterized by thickening of the glomerular basement membrane with absence of proliferation or signs of inflammation.

Therapy consisted of triamcinolone in doses varying from 20 to 40 mg./day. Duration of therapy ranged from one month to one year. None of the patients has had a complete remission. Persistent proteinuria from a trace to 6 Gm./day constituted the remaining manifestation in most cases. Protein excretion was reduced from a mean of 8 Gm. to 4 Gm./day/patient. Ten of the patients are edema free on mainten-

ance therapy. One patient improved only after withdrawal of the drug. Two died of thromboembolic disease while on therapy. Most of the patients developed Cushinoid features, and in 3 cases a profound, unexplained weakness with normal serum potassium was a problem. Five of the patients had been treated previously with prednisone. These cases and previous experience indicate that potency on a mg. per mg. basis is similar to prednisone. There was no evidence that triamcinolone promoted sodium excretion. Supplemental potassium therapy was not found necessary.

It is concluded that triamcinolone is as effective as prednisone or prednisolone in controlling the major manifestations of this disease, but it offers no advantages over these drugs. It has the disadvantage of producing unexplained weakness in some cases which was not seen with other steroids.

NEOPLASTIC DISEASE

Clinical Experiences with Mannitol Mustard (BCM or Degranol) in the Chemotherapy of Neoplastic Diseases: A Preliminary Report

By *R. J. Papac, N. L. Petrakis, W. A. Atchley, F. Amini and D. A. Wood.* Cancer Research Institute, University of California School of Medicine, San Francisco.

In the search for more effective alkylating agents, it has been reported that beta-dichlorodiethylamino and ethylenimino derivatives of sugars were among the most potent cytotoxic compounds in studies in animal tumors. Of the different combinations of sugars with various ethylenimino compounds, the most favorable chemotherapeutic properties occurred with 1,6-di-([2-chloroethylamino]-1,6-deoxy-D-mannitol dihydrochloride), known as mannitol mustard.

This compound has been administered to 14 patients with disseminated neoplastic diseases. It was administered intravenously as a saline infusion in doses ranging from 10 to 25 mg./Kg. The doses were given as single injections or in divided doses given over 7 days time. The administration of single large doses of mannitol mustard was followed by nausea and vomiting, which could be minimized by giving the dose in repeated small doses. Significant hematopoietic depression was observed in most patients receiving a total dose greater than 20 mg./Kg., except in those with lymphomatous diseases who appeared to be somewhat more susceptible.

Therapeutic benefit was observed in patients with malignant lymphoma, manifested by regression of lymphadenopathy and splenomegaly. Prompt reduction of massive splenomegaly also occurred in a patient with chronic granulocytic leukemia who had manifested no appreciable reduction in spleen size following therapy with Myleran, prednisone, or roentgen therapy to the spleen. No response was observed in patients with malignancies of the breast, ovary, urinary bladder, bowel, kidney and prostate, even when marrow depression was produced.

The results of the present preliminary study suggest that mannitol mustard has a chemotherapeutic spectrum similar to other alkylating agents and as yet appears to offer no practical clinical therapeutic advantage over nitrogen mustard.

Iodine Metabolism in Hydatidiform Mole and Choriocarcinoma

By *J. Thomas Dowling, Norbert Freinkel and Sidney H. Ingbar.* V. A. Center, University of California Medical Center, Los Angeles; Thorndike Memorial Laboratory, Second and Fourth (Harvard) Medical Services, Boston City Hospital, Boston; and Howard Hughes Medical Institute.

Rare but unique opportunities for study of the effects of gestation on iodine metabolism are provided by the pathologic pregnancies: hydatidiform mole and choriocarcinoma. The following parameters of thyroidal economy were assessed before and after treatment of 4 patients with these disorders: chorionic gonadotrophin (CG), 24-hour thyroidal I^{131} and I^{127} accumulation, concentrations of PBI^{127}, and SPI^{131} and BEI^{131} 24 and 72 hours after administration of the tracer. Saturation capacities of the circulating interalpha globulin (TBP), and the thyroxine-binding prealbumin (TBPA) for thyroxine were measured by methods developed in this laboratory.

In one preeclamptic molar pregnancy, 24-hour thyroidal accumulation of I^{131} was 82% and daily I^{127} incorporation, 2,147 μg. Concentrations of

PBI^{127} and PBI^{131} and BEI^{131} were similarly in the hyperthyroid range. Saturation capacity of TBP for thyroxine was 150 μg. (normal pregnancy range: 75-100 μg.), and of TBPA, 22 μg. (Normal: 90-120 μg.). Six weeks after hysterotomy, urinary CG disappeared and the several parameters of thyroid function were normal. Less striking but similar changes were noted in the 2nd patient with a molar pregnancy uncomplicated by toxemia. Changes in serum thyroxine-binding characteristic of pregnancy were not found in either patient with choriocarcinoma, in spite of high CG excretion and evidence for increased thyroidal hormonogenesis.

Extreme increase in thyroid hormone production in molar pregnancy may be added to the earlier observed exaggerated endocrine responses characteristic of the disease. Coupled with abnormal reduction in circulating TBPA in the patient with preeclampsia, these changes suggest a possible relationship of disturbed thyroidal economy to the pathogenesis of toxemia. Studies of 2 patients with choriocarcinomas demonstrated the independence of CG elaboration and changes in serum thyroxine–binding. Of further interest was the suggestion of increased thyroidal function in these individuals without altered interaction of thyroxine with circulating proteins.

A New Antitumor Agent, Streptovitacin A

By *John B. Field, Annie Mireles, Edward C. Dolendo, Francoise Costa, Angela Boryczka, Herbert Pachl, Louis Bascoy, Louis Cano and Weldon K. Bullock.* Department of Medicine, School of Medicine, University of Southern California, and Los Angeles County General Hospital, Los Angeles.

For a number of years this laboratory has studied antibiotics as sources of new agents to inhibit tumor growth. In the course of such a program a fermentation product of a Streptomyces was obtained which gave in mice with Crocker Sarcoma 180 a moderate but variable degree of tumor inhibition. An appraisal was undertaken of the remaining beer solids of the Streptomyces fermentation. Surprisingly, a suspension of the crude material given intraperitoneally at 300 mg./Kg./day reduced the tumors to 51% of the size of controls. In DBA mice with RC Carcinoma the tumor inhibition was 42%. These observations initiated the fractionation and characterization procedures which resulted in the isolation of a new antitumor principle, Streptovitacin A.

This agent has been evaluated under a variety of conditions in a spectrum of mouse and rat tumors. The latter include Sarcoma 180, RC Carcinoma, Ehrlich Ascites, Leukemia L 4946, Walker carcino-sarcoma, Jensen Sarcoma and Murphy-Strum lymphosarcoma. A significant inhibition has been obtained against all tumors studied and with relatively minute quantities of drug. With approximately 0.5 mg./Kg./day, the Sarcoma 180 was inhibited to about 60% of controls by all routes utilized. RC Carcinoma was inhibited to 41% of controls by 0.6 mg./Kg./day of the new agent; with the same dose Ehrlich Ascites tumor mice exhibited a prolongation of life of 56% beyond that of controls, and Leukemia L 4946 tumors were reduced to 47% of controls with 0.5 mg./Kg./day of Streptovitacin A. The rat tumors were inhibited in a manner similar to the mice. The tumors of treated animals showed marked disruption of normal growth with areas of liquefaction and necrosis. Evaluation in dogs with spontaneous tumors and early clinical trials has been initiated.

NERVOUS SYSTEM

Experimental Studies in Motivation

By *Roger C. Eddy, Wesley W. Wilson and Thomas H. Holmes.* Departments of Psychiatry and Physiology and Biophysics, University of Washington School of Medicine, Seattle.

An attempt was made to measure the behavior of albino rats stimulating themselves through permanently implanted intracranial electrodes. Measures used were food and water consumption, weight change and rate of bar-pressing response. No significant difference in daily weight change, food and water consumption was noted between animals on a daily self-stimulation schedule and operated controls. Animals were trained in an exploration habit in a 2-bar Skinner box. The stimulator was connected to a microswitch operated by the bar. One bar would actuate the stimulus in any experimental period. Learning the habit required the animal to press

both bars to find which one would actuate the stimulus. Six of 14 rats trained were able to make more than 75% of their response to the stimulus bar on 3 successive days. These rats were then tested to determine whether a preference existed for stimulus duration. A stimulator was connected to each bar and the rat allowed to select between 3 stimulus durations systematically assigned to the bars. Each animal showed a preference, but no consistent preference was found for the group. Variability in daily rate of bar-pressing response and observation of bar-pressing behavior by the experimenters suggest that the reinforcing effects of intracranial self-stimulation may be based upon more than one type of motivation. Animals in this series were found to have electrodes in the cerebral peduncles and subthalamic nuclei, as well as the ventromedial nucleus of the hypothalamus. Individual differences in bar-pressing patterns of different animals recorded by a pen-motor also suggest that important individual differences may exist in this behavior.

Cholesterol and Fatty Acid Synthesis in the Central Portion of Cut Sciatic Nerve

By *Ann H. Hughes and Sven G. Eliasson.* Department of Internal Medicine, University of Texas Southwestern Medical School.

It has been previously established that cholesterol synthesis in the normal peripheral nerve takes place in the connective tissue elements and Schwann cells, while fatty acid synthesis occurs primarily in the neural elements. The purpose of this investigation is to determine localization of cholesterol and fatty acid synthesis in sectioned peripheral nerve.

Unilateral sections of cat sciatic nerves were made and after varying time intervals proximal segments from the cut side and comparable segments from the intact side were removed and homogenized. Cholesterol and fatty acids were isolated and assayed after the neural and connective tissue elements were incubated with acetate-I-C^{14}.

It was found that the homogenates of the cut and uncut nerves synthesize a negligible amount of cholesterol. Furthermore the cholesterol synthesis in the sheath from the cut side exceeds that of the homogenates and the sheath from the control side. Segments proximal to the cut ends were also found to synthesize very small quantities of cholesterol.

The relative increase in cholesterol synthesis in the sheath of the cut nerve reaches the level of cholesterol synthesis in sciatic sheaths from non-operated animals. Therefore, there is actually a decrease in synthesis in the proximal and control segments, which we postulate to be due to a release of inhibitory factors, possibly nucleotidases.

Fatty acid synthesis was found to take place in a random fashion in the homogenates and only to a lesser extent in the sheaths. There is usually a higher level of synthesis on the cut side. We feel that the explanation for the variability in fatty acid synthesis lies in the appearance of variable numbers and selective myelinisation of sprouting nerve fibers.

PHARMACOLOGY AND THERAPEUTICS

A Study of the Anticholinesterase Properties of EPN and Malathion in Human Volunteers

By *J. Alfred Rider, Hugo C. Moeller, Joyce Swader and Richard G. Devereaux.* Gastrointestinal Clinic, Department of Medicine, University of California School of Medicine, San Francisco.

EPN and malathion are 2 of the most widely used organic phosphate anticholinesterase insecticides in agriculture. Although the minimum tolerated dose of these agents has been established in animals, no comparable studies have been made in humans. Since toxicity varies considerably among different animal species, it is important to establish by objective means the minimum tolerated dose for humans.

The purposes of this study were to determine (1) the amount of EPN and malathion that can be ingested daily by humans without side effects or significant depression of RBC and plasma cholinesterase, and (2) whether potentiation occurs when the two are given in combination.

The initial studies on 10 humans established that ingestion of 3 mg. EPN or 8 mg. malathion daily for 32 days did not depress plasma or RBC cholinesterase, and that 3 mg. EPN and 8 mg. malathion combined for 44 days produced no

side effects. A further group of 5 volunteers were given 6 mg. EPN daily for 88 days; during the last 44 they also received 8 mg. malathion daily. Another 5 volunteers took 16 mg. malathion daily for 88 days; during the last 41 they also received 3 mg. EPN. All 10 were then given 6 mg. EPN and 16 mg. malathion daily for 42 days.

The results indicate that 6 mg. EPN plus 8 mg. malathion or 3 mg. EPN plus 16 mg. malathion can be given to humans for prolonged periods without causing significant depression of RBC or plasma cholinesterase. However, 6 mg. EPN plus 16 mg. malathion per day depresses both RBC and plasma cholinesterase. This depression is slight and causes no symptoms. In the doses employed, no potentiation of EPN by malathion or of malathion by EPN was noted.

C^{14}-Chlorothiazide Studies in Human Beings

By *Herbert R. Brettell, Gerald S. Gordon and Jerry K. Aikawa.* Department of Medicine, University of Colorado School of Medicine, Denver.

3-C^{14}-tagged chlorothiazide was used as a tracer to study the metabolism of Diuril in human beings; 50 to 100 μc. of C^{14}-chlorothiazide was given orally or intravenously with 0.5 to 1.0 Gm. of the untagged material, and serial blood and urine specimens were obtained. Radioactivity was assayed with a windowless flow counter.

In 3 normal individuals given 0.5 Gm. of chlorothiazide orally, maximum serum concentration of 4 μg./ml. was obtained at 2 hrs. The cumulative renal excretion at 24 hrs. ranged between 33 and 58%, excretion rate being highest during the first 5 hrs.; 4 normal subjects given 1 Gm. orally showed a peak serum level at 3 hrs. of 10 μg./ml. The cumulative renal excretion ranged at 4 hrs. between 27 and 41%; at 8 hrs., 32 to 46%; and at 24 hrs., 35 to 49%. In 3 subjects given 0.5 Gm. intravenously, 90% of the radioactivity was excreted within 2 hrs., with complete recovery within 24 hrs.

In 4 patients with congestive heart failure, 1 showed a normal excretion pattern. The other 3 demonstrated decreased excretion rates or cumulative values. A compensated cirrhotic had a normal excretion pattern; 3 with decompensation showed impaired excretion. Two patients with chronic glomerular disease showed high blood levels with low excretion, while 1 subject with chronic pyelonephritis showed a normal pattern. One of the above patients with chronic glomerular disease was restudied with an intravenous dose. Excretion rate and cumulative percentage were low.

Impaired excretion of C^{14}-chlorothiazide was observed in patients with congestive heart failure, severe hepatic and renal glomerular diseases.

A Controlled (Placebo) Study of the Antihypertensive Effect of Chlorthiazide (Diuril)

By *Fred T. Darvill, Jr.* Northern State Hospital, Sedro-Woolley, Washington, and Department of Medicine, University of Washington School of Medicine, Seattle.

To assess antihypertensive effects, chlorthiazide and an identical placebo were administered to 21 patients with sustained diastolic hypertension over 100 mm. of mercury. Six patients (Group I) received no other medication. The previous antihypertensive medication of 15 patients (Group II) was reduced sufficiently to allow a rise in diastolic pressure ranging between 100 and 110 mm. Hg. After stabilization, constant doses of these medications (reserpine, hydralazine and mycamylamine) were continued throughout this study.

The drugs were administered for 12 weeks as follows: weeks 1 and 2, control period; weeks 3 and 4, placebo twice a day; weeks 5 to 8, chlorthiazide 0.5 Gm. twice a day; weeks 9 and 10, placebo twice a day; weeks 11 and 12, chlorthiazide 0.5 Gm. twice a day. Blood pressure determinations were made 3 times a day throughout this study. Mean diastolic values were obtained during weeks 2, 4, 5, 8, 9 and 11, and were subjected to statistical analysis.

Statistical evaluation confirmed the clinical impression that the drug, when given alone, exhibited significant consistent antihypertensive effects in 1 of the 6 patients studied (Group I); when given in combination with other antihypertensive drugs, 6 of the 15 patients (Group II) studied responded with significant consistent falls in diastolic pressure. However, these effects were not predictable in individual patients.

Interaction effects were significant in both groups at $p < .001$, confirming the clinical feeling that drug effect was not consistent in randomly chosen patients. Because of these findings, it was impossible to determine which of the group means was significantly different, if any.

It may be concluded that chlorthiazide, although weakly antihypertensive when administered alone, will frequently, although unpredict-

ably, potentiate the effect of other antihypertensive drugs. Responders could not be separated from non-responders on the basis of any clinical determinations.

Double Blind Evaluation of Methyl-Phenylpiperdylacetate (Ritalin) in the Management of Institutional Geriatric Patients

By *Fred T. Darvill, Jr.* Northern State Hospital, Sedro-Woolley, Washington, and Department of Medicine, University of Washington School of Medicine, Seattle.

Ritalin has been reported of value in managing committed geriatric patients, but the methodology of all studies to date may be criticized (i.e., small series, no controls, or no double blind technic).

Seventy committed senile patients (average age 78 years), randomized solely by case number, were given Ritalin (10 mg. tablets) or an identical placebo in a double blind manner.

Thirty patients (Group I) received one tablet daily of Ritalin or placebo the 1st week, 2 the 2nd week, and 3 the 3rd to 6th weeks. Forty patients (Group II) received one tablet daily the 1st week and thereafter weekly increments of one tablet daily for the next 5 weeks. Both groups were observed off medication for 2 additional weeks.

Before treatment, and thereafter weekly for 8 weeks, recordings of pulse, blood pressure, weight and medical complications were made; additionally, orientation, appetite, continence, cooperation, activity, rationality, personal care and irritability were scored as follows: 2 plus—much improved, 1 plus—some improvement, 0—no change, minus 1—somewhat worse, and minus 2—much worse. Final over-all evaluation was made on the same scale. Scoring was done in conference by the investigator, ward physician, ward nurse and attendants.

In Group I, 5 patients improved (three, 1 plus; two, 2 plus) on Ritalin, and 7 patients improved on placebo (five, 1 plus; two, 2 plus); 2 became worse (both minus 1) on Ritalin and 1 became worse (minus 1) on placebo. In Group II, 6 patients improved (four, plus 1; two, plus 2) on Ritalin, and 7 patients improved (six, plus 1; one, plus 2) on placebo. Two became worse (one, minus 1; one, minus 2) on Ritalin and 3 became worse (all minus 1) on placebo. No significant toxicity was encountered.

In conclusion, no significant differences were noted between Ritalin and the placebo in this study.

RESEARCH METHODS

Electrodes for Blood pO_2 and pCO_2 Determination

By *John W. Severinghaus.* Cardiovascular Research Institute, University of California, San Francisco.

Direct electrical measurement of blood oxygen and carbon dioxide partial pressure has recently been made possible by the development and application of special electrodes. The Clark polarographic electrode is a platinum disc charged to −0.5 volts in an electrolyte, KCl, separated from the blood by an oxygen permeable membrane, such as polyethylene. Oxygen molecules react at the platinum forming H_2O_2 and OH^-. This results in current which is linearly related to pO_2. It has been found necessary to stir the blood at constant rate at the electrode surface, and to calibrate the electrode with blood and gas of the same pO_2 is about 10%. A 0.4 ml. cuvette of stainless steel has been designed, incorporating a tiny stirring paddle, and built into a 1 L. water bath. The water bath also contains a tonometer for preparing equilibrated blood for calibrating the oxygen electrode. A transistorized null balance current measuring device has been built, having a reading accuracy of 0.1% The CO_2 electrode operates by measuring the pH of a film of dilute bicarbonate solution which is separated from the blood by a CO_2 permeable membrane such as Teflon. The pH of the aqueous film is linearly related to the log of the pCO_2 in the blood over the range from 1% to 100% CO_2. The electrode is built into a 0.3 ml. cuvette which does not require stirring, and is not viscosity or pressure sensitive. It has been mounted in the same water bath with the pO_2 electrode. 0.01 pH change represents about 2.5% change in pCO_2. The response time is 20 seconds to 2 minutes, being more rapid at high pCO_2, and the electrode is calibrated with gas of known pCO_2 directly in the cuvette. It requires a high sensitivity pH meter.

RESPIRATORY SYSTEM

The Effect of Chest Strapping on Cardiopulmonary Function

By *M. B. McIlroy, J. Butler and T. Finley.* Cardiovascular Research Institute, University of California Medical Center, San Francisco.

The effect of strapping the chest with a length of rubber has been studied in normal subjects. There is a reduction in lung compliance, an increase in respiratory rate and a reduction in functional residual capacity. Some reduction in arterial oxygen saturation occurs; this can be temporarily abolished by a full inspiration, which also restores the mechanical properties of the lungs to normal.

The changes in cardiopulmonary function which have been observed indicate that chest strapping reduces the alveolar surface available for gas exchange by closing down parts of the lung and producing, among other things, increased shunting across the lungs.

The physiologic situation produced by chest strapping resembles that found in massive collapse of the lung and pneumothorax.

Erythrokinetics in Chronic Hypoxemic Pulmonary Emphysema

By *Armand P. Gelpi, James N. Castle, William A. Reilly and Gilbert L. Searle.* San Leandro, California, and V. A. Hospital, San Francisco.

The purpose of this investigation was to uncover factors which may be responsible for the apparent infrequency of secondary polycythemia in patients with hypoxemic pulmonary emphysema. A secondary aim was to determine in such patients if patterns of erythrokinetics and iron utilization resembled those seen with chronic infection.

Eight patients with advanced pulmonary emphysema, normal or slightly elevated hemoglobin concentrations, and variable degrees of hypoxemia had complete hematologic studies, including bone marrow examinations and serum iron determinations. All patients had iron turnover studies, using Fe^{59}; 7 patients had Cr^{51} red cell survival studies, and 6 patients had blood volume and total red cell mass estimates.

Six patients showed an increase in iron turnover above normal, and 6 patients showed shortened red cell life spans. These findings could not be correlated with other indices of erythropoietic activity, with the degree of hypoxemia or with variations in blood volume or total circulating red cell mass. Wide variations in serum iron concentration were observed, correlating roughly with marrow iron content: with low serum iron there was little or no marrow iron present. With elevated serum iron there appeared to be increased marrow iron. None of the patients had reduced serum iron–binding capacity. Venous hematocrits were uniformly and disproportionately increased above normal as compared with corresponding hemoglobin concentrations. Increased marrow erythroid activity and reticulocytosis was observed in only one patient, who had marked hypoxemia. Red cell Fe^{59} utilization was unexpectedly impaired in 3 of the patients.

The data suggest that in patients with hypoxemic pulmonary emphysema there is an incomplete and unpredictable erythropoietic response associated with a decreased red cell survival. There is no evidence to suggest that these patients have a defect in iron utilization comparable to that seen in patients with chronic infection.

Bronchial Collapse in Pulmonary Emphysema: a Morphologic Study

By *Robert R. Wright.* Department of Pathology, University of California, School of Medicine, San Francisco.

The purpose of this study was to demonstrate the morphology of the expiratory obstruction which occurs in chronic obstructive pulmonary emphysema.

Autopsied lung specimens were fixed by infiltration through the main bronchus with 10% neutral formalin until the normal contours of the lobes were established. The bronchial trees of 12 emphysematous lungs and 12 normal lungs were dissected free of the parenchyma and compared by gross and microscopic study.

There was a marked difference between the bronchi of the emphysematous lungs as compared to the normal. In all cases, atrophy of the bronchi distal to approximately the 3rd order was demonstrated. This was evidenced by thinning and ectasia of the walls, decreased amounts of supporting cartilage, loss of smooth muscle

and connective tissue, and a grossly apparent decreased resistance to collapse. Active inflammation was not seen in the cases studied.

The normal intrapulmonary and intrabronchial pressure relationships are such that there is a tendency for the bronchi to collapse during a forced expiration. This is offset by the support of the bronchial wall with cartilage rings and plates, connective tissue, the spiral smooth muscle bundles and the elastic retracting forces of the pulmonary parenchyma. When the normal lung is sufficiently deflated, the smaller bronchi and bronchioles collapse as they are supported mainly by the elastic parenchyma. This degree of deflation leaves a relatively small amount of air within the normal lung. The emphysematous lung, however, shows a premature collapse of the atrophic poorly supported bronchi and bronchioles during the expiratory phase of ventilation and especially during a forced expiration. This causes an obstruction to the outflow of air with resultant "air trapping" in the lung.

PROGRAM

Western Society for Clinical Research

Thursday, Friday and Saturday, January 29, 30 and 31, 1959
The Golden Bough Theater, Carmel, California

Dr. Herbert N. Hultgren, Presiding

THURSDAY, JANUARY 29

2:00 P.M.

The President's Address: Herbert N. Hultgren, M.D.

1. **The Effect of the Acute and Chronic Administration of Hydrocortisone on the Release, Inactivation and Action of Antidiuretic Hormone (ADH).**
   Charles R. Kleeman, Jerry Koplowitz, Morton H. Maxwell* and J. Thomas Dowling,** Los Angeles. *page 111*
2. **Steroidogenesis by Adrenal Adenomata and Nontumorous Adrenal Tissue in Vitro.**
   C. I. Slade, R. E. Bailey,* A. H. Lieberman and J. A. Luetscher, Jr.*, San Francisco. *page 112*
3. **Preliminary Observations on Prolactin Activity in Human Blood.**
   *Benjamin Simkin and David Goodart,** Los Angeles. *page 107*
4. **Fatty Oxidation in Man as Affected by Nutritional States.**
   *Josiah Brown and Leslie R. Bennett,** Los Angeles. *page 116*
5. **Evidence for Control of Unesterified Fatty Acid Metabolism by the Sympathetic Nervous System.**
   Richard J. Havel and Alan Goldfien, San Francisco. *page 116*
6. **The Renal Excretion of Uric Acid in Patients with Gout and in Nongouty Subjects.**
   C. A. Nugent and F. H. Tyler*, Salt Lake City. *page 113*
7. **A Study of Granulocyte Kinetic Models.**
   John W. Athens, Alvin M. Mauer, Homer R. Warner, Helen Ashenbrucker,* George E. Cartwright and Maxwell M. Wintrobe*, Salt Lake City. *page 92*
8. **Biochemical Genetics of Glucose-6-Phosphate Dehydrogenase Deficiency.**
   A. G. Motulsky, J. M. Kraut, W. T. Thieme* and D. F. Musto,** Seattle. *page 89*
9. **Development of Pulmonary Hypertension in Mitral Stenosis.**
   Arthur Selzer, San Francisco. *page 101*
10. **The Effect of Heart Rate on Cardiac Output and Arterial Pressure in Dogs at Rest and during Exercise.**
    Homer R. Warner, Cloyd C. Hofheins and Alan F. Toronto,** Salt Lake City. *page 100*
11. **The Reiter Protein Complement Fixation (RPCF) Test in the Diagnosis of Syphilis.**
    James N. Miller, Ruth A. Boak* and Charles M. Carpenter*, Los Angeles. *page 123*
12. **Bacterial Polysaccharide Binding with Plasma Proteins.**
    Russell S. Jones, Salt Lake City. *page 122*

FRIDAY, JANUARY 30

9:00 A.M.

Monte Greer, M.D., Presiding

13. **Some Observations on the Treatment of Post-irradiation Hematopoietic Depression in Man by the Infusion of Stored Autogenous Bone Marrow.**
    N. B. Kurnick, Bernard H. Feder, James C. Gerdes* and Andrew Montano,** Los Angeles and Long Beach. *page 93*
14. **Clotting Defects in the Dysproteinemias and Paraproteinemias.**
    Seymour Perry, William A. Skoog and William S. Adams, Los Angeles. *page 95*
15. **The Estimation of Erythrocyte Survival Time in Hemolytic Disease from a Single Sample of Blood.**

*By Invitation
**Emeritus Member

Jean C. Sabine, Harry Lee,* Catherine A. Lambert* and Myron Pollycove,** San Francisco and Berkeley. (Introduced by *N. L. Petrakis.*) *page 90*

16. **The Serum Haptoglobins: Some Clinical and Genetic Studies.**
Eloise R. Giblett, Seattle. *page 92*

17. **Metabolic Changes Produced by Human Growth Hormone (Li) in a Pituitary Dwarf.**
Roberto F. Escamilla, John J. Hutchings,* William C. Deamer* and Choh Hao Li,** San Francisco and Berkeley. (Introduced by *Peter H. Forsham.*) *page 107*

18. **Metabolic Changes Produced by Chymotrypsin Digests of Bovine Somatotropin in Man.**
Vincent C. DiRaimondo, Ernest Gold, Stanley Newman,* Rex Bigler,* Felix O. Kolb, Choh Hao Li* and Peter H. Forsham,* Berkeley. *page 107*

19. **The Acute Effects of DBI on Human Hepatic Intermediary Metabolism.**
Robert Tranquada, Charles Kleeman and Josiah Brown,* Los Angeles. *page 110*

20. **Tissue Distribution of Administered DBI and Its Relationship to DBI Action.**
*Arne N. Wick and Charles J. Stewart,** San Diego. *page 111*

21. **Comparative Analysis of I^{131} Albumin Metabolism and Distribution.**
Warren L. Beeken, Wade Volwiler, Patrick D. Goldsworthy,* Patricia Ann Wood,* Marion P. MacMartin* and Chester Westort,** Seattle. *page 114*

22. **Amino Acid Patterns in Arterial and Hepatic Venous Blood.**
Sherman M. Mellinkoff, Telfer B. Reynolds, Marjorie Frankland and Margaret Greipel,** Los Angeles. *page 117*

23. **Evidence for the Metabolism of Bromsulfalein (BSP).**
John V. Carbone, G. M. Grodsky and R. Fanska,** San Francisco. *page 118*

24. **Effects of Bromsulfalein on the Cardioportal Circulation Time.**
Ismael Mena, Leslie R. Bennett* and R. Wilbur Melbye,** Los Angeles. (Introduced by *Joseph F. Ross.*) *page 119*

FRIDAY, JANUARY 30
ALTERNATE SESSION
9:00 A.M.

Albert A. Kattus, Jr., M.D., Presiding

25. **Reversal of Quinidine-induced Retardation of Myocardial Depolarization by Sodium Lactate and Hyperventilation.**
H. Lenox H. Dick and Elton L. McCawley, Portland. *page 97*

26. **Relationships Between Stroke Volume, Cardiac Output and "Central Blood Volume" in Cardiac Patients.**
Leonard A. Cobb, Lewis A. Ralston* and Robert A. Bruce,* Seattle. *page 99*

27. **Central Circulatory Changes Accompanying Mitral Commissurotomy.**
Harold G. Richman, J. Bradley Long* and Elliot Rapaport. page 101*

28. **Lack of Correlation between Myocardial Oxygen Tension and the Electrocardiogram.**
Naci Bor and Peter F. Salisbury,* Burbank. *page 98*

29. **The Nature of Acute ST Changes in Experimental Coronary Occlusion.**
Werner E. Samson and Allen M. Scher,** Seattle. (Introduced by *R. A. Bruce.*) *page 97*

30. **Uterine and Fetal Blood Flow and Oxygen Consumption in Early Human Pregnancy.**
N. S. Assali, L. Rauramo, T. Peltonen* and C. Gemzell,** Los Angeles, Turku, Finland, and Stockholm, Sweden. *page 121*

31. **A Comparison of Pulmonary Physiologic Studies with Gough Sections of Postmortem Lungs.**
*Jerome E. Cohn, Terence H. Cochran, Charles T. Pinney,** Salt Lake City. *page 128*

32. **The Influence of Mechanical Factors on the Respiratory Work and Ventilatory Responses to Carbon Dioxide.**
*Frederic Eldridge and John Davis,** San Francisco. *page 127*

33. **Freely Extractable Plasma Lipid.**
George D. Michaels, Paul F. Flynn, Geoffrey Walker,* Adolpho Barcellini* and Laurance W. Kinsell,* Oakland. *page 115*

34. **Variation in Serum Lipids during Mental and Emotional Stress.**
P. T. Wertlake, A. A. Wilcox,* M. I. Haley* and J. E. Peterson,* Loma Linda. *page 105*

35. **Effect of Stress on Rats Fed a High Fat Diet in the Development of Atherosclerosis.**
Herman N. Uhley and Meyer Friedman,* San Francisco. *page 105*

36. **Lipide Synthesis in Normal and Obese Mice.**

D. D. Feller, E. Feist,* R. L. Huff and N. R. Eaton,** Seattle. *page 115*

12:00 Noon

BUSINESS MEETING

SATURDAY, JANUARY 31

9:00 A.M.

Robert Aldrich, M.D., Presiding

37. **Immunologic Studies in the Connective Tissue Diseases.**
W. J. Fessel, Wallace V. Epstein and Ephraim P. Engleman,* San Francisco. *page 121*

38. **Studies on a Wasting Disease Induced in Hybrid Mice Injected with Parental Strain Lymphoid Cells.**
H. S. Kaplan, B. H. Rosston and R. Skahen,** San Francisco. *page 121*

39. **Hypovolemic Shock and Hypotension in the Nephrotic Syndrome.**
Hiroshi Yamauchi and James Hopper, Jr.,* San Francisco. *page 125*

40. **Induced Cardiac Arrest as an Aid to Angiocardiography.**
John C. English, William S. Hoskinson,* Louis H. Frische* and Charles T. Dotter,* Portland. *page 103*

41. **Effects of Partial and of Total Heart-Lung Bypass on the Heart.**
*Peter F. Salisbury and Naci Bor,** Burbank. *page 100*

42. **Effects of Clinical Digitalis Levels on Rubidium Uptake of Red Blood Cells.**
Jerold M. Lowenstein, William L. Caldwell* and Grover Liese,** San Francisco. (Introduced by *Paul Aggeler.*) *page 103*

43. **The Ventilatory Response of SCUBA Divers to CO_2 Inhalations.**
*Herman F. Froeb,** La Jolla. (Introduced by *W. P. Vanderlaan.*) *page 127*

44. **Pernicious Anemia due to the Presence of Intrinsic Factor Inhibitor Diagnosed in Childhood, with a 25-Year Follow-up.**
*Jonah G. Li, Stacy R. Mettier,** Harold A. Harper* and Alice McBride,** San Francisco. *page 90*

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BLOOD

Studies on the Enzymic Synthesis of Heme from Iron and Protoporphyrin

By *H. C. Schwartz, R. L. Hill, G. E. Cartwright and M. M. Wintrobe.* Departments of Medicine and Biological Chemistry, University of Utah College of Medicine, Salt Lake City.

An attempt has been made to purify the enzyme system in chicken erythrocytes that is responsible for the biosynthesis of heme from iron and protoporphyrin. Heme synthesis was measured as the % incorporation of Fe^{59} into heme after incubation of the following: an hemolysate or a purified fraction of an hemolysate, 5×10^{-5} M protoporphyrin, 1×10^{-3} M cysteine, 1×10^{-2} M Tris buffer, carrier iron and radioiron. In previous studies, we have demonstrated that cysteine or reduced glutathione is necessary for maximal activity.

Fractionation procedures, designed to remove hemoglobin from an active, 0.15 M KCl extract of a water hemolysate, resulted in complete loss of enzyme activity. The procedures used included various concentrations of chloroform-ethanol, 20% chloroform, 20% n-butanol, acetone (−60 C.) precipitation, selective heat denaturation and ammonium sulfate fractionation.

Successful purification was achieved in the following manner: An 0.03% saponin hemolysate was centrifuged, and the active particulate matter was separated from the inactive supernatant solution (A). The particulate matter was washed free of hemoglobin with 0.15 M KCl, homogenized in a Waring blendor and centrifuged. The resulting supernatant solution (B) showed a maximal 40-fold purification, calculated on the basis of units of activity per mg. of nitrogen, with a unit yield of 20%. Marked variability in the activity of different purified preparations suggested that an essential factor was being lost in the washes. In 3 experiments, reconstitution of the supernatant solution (B) with the inactive supernatant solution (A) resulted in a 2- to 15-fold augmentation in enzyme activity. Thus, enzymic activity in the hemolysate is due to 2 or more factors, one present in the particulate fraction and the other in the supernatant fluid.

Biochemical Genetics of Glucose-6-Phosphate Dehydrogenase Deficiency

By *A. G. Motulsky, J. M. Kraut, W. T. Thieme and D. F. Musto.* Department of Medicine, University of Washington, Seattle.

Investigations were undertaken to elucidate the nature and significance of the inherited erythrocyte glucose-6-phosphate dehydrogenase deficiency which predisposes to hemolytic anemia from some drugs and fava beans.

The geographic limitation of the trait to many subtropical and tropical areas suggests a possible selective advantage of the defect in that environment. Nonprotein glutathione is diminished and easily depleted in enzyme-deficient cells. Since malarial organisms require glutathione for growth, enzyme deficient subjects may be more resistant to falciparum malaria.

A rapid test for enzyme deficiency was developed, using brilliant cresyl blue as indicator for the enzyme-catalyzed reaction. Blood specimens of normal males decolorized in 45-90 minutes (majority: 50-75 minutes), while enzyme deficient subjects required 140 minutes to 29 hours (majority: 3-5 hours). The test is suitable for hospital and genetic surveys.

Ten % of male Negroes and 10% of Asiatic Indians were found to be enzyme deficient. Genetic studies were compatible with sex-linked inheritance. The expected frequency of female heterozygotes was not found, indicating that female heterozygotes frequently have normal enzyme activity. Some female heterozygotes gave intermediate results.

The pH optimum of the enzyme of deficient males was lower than that of normals. Similarly, heat lability differed in abnormals. It is suggested that "enzyme-deficient" subjects have qualitatively altered enzyme structure with defective activity rather than quantitative enzyme lack. The basic mutation probably affects protein structure in a way analogous to the defect in the hemoglobinopathies.

The Estimation of Erythrocyte Survival Time in Hemolytic Disease from a Single Sample of Blood

By *Jean C. Sabine, Harry Lee, Catherine A. Lambert and Myron Pollycove.* Cancer Research Institute and Departments of Medicine and Radiology, University of California Medical Center, San Francisco, and Donner Laboratory, Berkeley.

In patients with accelerated erythropoiesis in response to hemolytic disease, mean cell age should correspond to mean cell survival time. The erythrocyte cholinesterase activity (RBC ChE) affords a means of estimating cell age, without the use of radioisotopes or the injection or reinjection of any material. Technically, the determinations are not difficult nor is elaborate equipment required.

Comparison has been made of the RBC ChE and the mean cell survival time estimated by Cr^{51}-tagging or by Fe^{59} turnover. Data for 16 patients were compatible with a calculated curve expressing the hypotheses that cholinesterase activity decreases exponentially with cell age, that on zero day the activity is 3 times the mean activity of a normal cell population aged zero to 120 days, and that in hemolytic disease cell destruction occurs at random. In 5 cases, the survival time could not be estimated from the RBC ChE which was low, confirming clinical evidence that erythropoietic response was poor or absent.

Two additional observations tend to confirm the validity of the hypotheses mentioned above.

The distribution of RBC ChE in 52 cases of hemolytic disease indicates that there are 2 populations, one with low values, one with high. There were no values in the midnormal region where the distribution of normal values calls for 23.

Patient's cells were divided by centrifugation into layers. In patients with markedly accelerated cell production and destruction, the top (reticulocyte-rich) layer had RBC ChE approaching 3.0, the suggested value for new cells. The RBC ChE of the layers decreased from top to bottom, and the data were compatible with predicted curves.

In interpreting the RBC ChE, the observer must decide whether in the individual patient cell age corresponds to cell survival time. Stimuli to accelerated erythropoiesis other than the destruction of cells in the body must be ruled out. Recent transfusion results in a titer which is the addition product of the RBC ChE of the patient and of the donor.

Pernicious Anemia due to the Presence of Intrinsic Factor Inhibitor Diagnosed in Childhood, with a 25-Year Follow-up

By *Jonah G. Li, Stacy R. Mettier, Harold A. Harper and Alice McBride.* Departments of Medicine and Surgery, University of California School of Medicine, San Francisco.

An unusual case of pernicious anemia diagnosed in a male at the age of 13 months with a 25-year follow-up is reported.

Free acid and extremely high levels of urinary uropepsin were consistently found. The anemia responded to intramuscular injection of B_{12}, but not to oral administration of the vitamin together with intrinsic factor. Radioactive vitamin B_{12} studies demonstrated extremely poor intestinal absorption. This defect was not influenced by the addition of intrinsic factor, normal gastric juice or gastric juice from a patient known to have pernicious anemia. Massive dosage of intrinsic factor given orally did not correct the absorptive defect. Sterilization of the

intestinal tract with tetracycline before administration of vitamin B_{12} also failed to improve the absorption. Fifty ml. of the patient's gastric juice plus intrinsic factor did not inhibit the intestinal absorption of vitamin B_{12} in a patient with polycythemia. Five hundred ml. of the patient's gastric juice plus vitamin B_{12} and intrinsic factor given orally to a woman with a subtotal gastrectomy and with normal B_{12} absorption depressed her absorption of B_{12} by 50%.

Therefore, is is concluded that this patient's gastric juice, in sufficient quantity, will inhibit the absorption of B_{12} from the intestinal tract, causing a variant of pernicious anemia.

Effect of Inosine on Stored Rabbit Blood

By *A. William Shafer and Grant R. Bartlett.* Scripps Clinic and Research Foundation, La Jolla, California.

Certain nucleosides, notably adenosine and inosine, have been reported approximately to double the time blood could be stored. Recent reports on this effect have been conflicting, and the present studies were designed to give more information concerning the influence of inosine on the stored red blood cell.

Rabbit blood was kept for 6 weeks at 4 C. in ACD or in ACD plus inosine. The post-transfusion survival was determined by labeling the stored red cells with Cr^{51} and by using P^{32}-labeled fresh red cells for blood volume assay.

Blood stored for 6 weeks in ACD alone showed 10 to 25% survival of the red cells 24 hours after transfusion. In the first experiment, post-transfusion survival exceeded 70% for all samples stored 6 weeks with inosine, whether added initially, semiweekly or at the end of 3 weeks of storage. Subsequent results were more variable, and the post-transfusion survival of ACDI-stored blood ranged from 50-90%. Periodic shaking of the blood during storage, or autoclaving the inosine in ACD or in saline made no difference on the survival. Various lots of inosine were tested, and all seemed to be equally effective. In confirmation of previous reports inosine had a striking effect on maintaining the red cell organic phosphates.

Although the question of variability in the inosine effect is still unresolved, several possibilities have been eliminated as modifying factors; and it has been confirmed that inosine has a markedly favorable influence on extending the storage life of the rabbit red cell.

Studies on the Selective Labeling by Sodium Chromate (Cr^{51}) of the Separated Components of Adult Hemoglobin by Starch Block Electrophoresis

By *Nicholas L. Petrakis, Marie Doherty and Salvatore P. Lucia.* Department of Preventive Medicine, Department of Medicine, and Cancer Research Institute.

It recently has been demonstrated that adult hemoglobin can be separated into several components by means of starch block electrophoresis. The fractions consist of a major component A_1, a slow-moving minor component A_2, a fast-moving minor component A_3, as well as several less well-defined components. The present studies were undertaken to determine the distribution of Cr^{51} among the various adult hemoglobin components separated by starch block electrophoresis.

Venous blood from normal subjects and patients was tagged with Cr^{51} in the manner employed for in vivo erythrocyte survival determinations. The labeled cells were washed 3 times with normal saline, and hemolysates were prepared according to the method of Singer. The electrophoretic separation was carried out on potato starch, using the method of Kunkel, employing a phosphate buffer pH 8.6, ionic strength 0.05. The time of separation averaged from 24 to 48 hours in a field of 400 volts and 30 milliamperes at 4 C. The starch block containing the hemoglobin fractions was then cut into sections, eluted with distilled water, and the hemoglobin concentrations and radioactivities of the eluates were determined. Electrophoretic preparations of nonlabeled hemoglobin were employed as controls.

It was found that the Cr^{51} was present in the A_1 and the A_3 components. The A_3 component, comprising approximately 4% of the total hemoglobin, contained on the average 46% of the sodium chromate. The A_1 component, comprising 94% of the hemoglobin, contained on the average 54% of the label. The A_2 component, comprising 2% of the total hemoglobin, did not contain significant amounts of the label. Control studies of unlabeled hemoglobin indicated that the labeling process did not appear to affect the electrophoretic migration of these components.

These preliminary findings indicate that the components of adult hemoglobin differ in their capacity to bind sodium chromate-51. Work is in progress to extend these observations in the

study of the hemoglobinopathies and the physiologic activities of young and old erythrocytes.

The Serum Haptoglobins: Some Clinical and Genetic Studies

By *Eloise R. Giblett*. King County Central Blood Bank, and Department of Medicine, University of Washington School of Medicine, Seattle.

Electrophoresis of serum in starch gel was shown by Smithies to result in separation of the alpha and beta globulins into genetically controlled components that are not seen when other technics are used. The present study was designed to investigate methods of qualitation and quantitation of the haptoglobins (those alpha-2 globulins which bind hemoglobin); to determine racial distribution of the haptoglobin types; and to demonstrate starch gel electrophoretic patterns associated with certain hematologic disorders.

Comparison of various buffers revealed maximum resolution with Poulik's tris-borate discontinuous buffer system. This system was used to test 400 serum specimens from Negroes and 200 from non-Negroes. Whereas the expected distribution of haptoglobin types was found in the non-Negro group, the Negro sera showed a considerable difference from the reported incidence, as well as 3 modifications rare or absent in non-Negroes.

Haptoglobin quantitation was achieved by the addition of known amounts of hemoglobin to serum prior to electrophoresis on paper or starch gel. The presence of free hemoglobin was readily detected when haptoglobin saturation was exceeded. This method was employed to measure haptoglobin turnover time in a patient with multiple myeloma whose hemoglobin-binding protein was markedly increased.

Greatly diminished or absent haptoglobin secondary to red cell destruction was characteristic of patients with various types of hemolytic anemia. One subject with "march hemoglobinemia" had only trace amounts of serum haptoglobin following several weeks of inactivity, suggesting that his reduced hemoglobin-binding capacity was contributory to hemoglobinuria following exercise.

Human Leukocyte Alkaline Phosphatase: Studies with Zinc and Magnesium

By *Kouichi R. Tanaka and William N. Valentine*. Department of Medicine, School of Medicine, University of California Medical Center, and V. A. Center, Los Angeles.

Human leukocyte alkaline phosphatase activity exhibits marked lability in disease. Magnesium is regarded as the classical primary metal activator, but investigations elsewhere on the possible role of zinc prompted the following study of metal activation. Activity is expressed as mg. of phosphorus liberated from sodium beta-glycerophosphate at pH 9.9 by 10^{10} W.B.C. per hour at 37 C.

High activity leukocytes (>75) exhibited progressive loss of phosphatase activity on saline incubation averaging 21, 31 and 42% after 1, 2 and three hours, respectively. In lower activity populations (10-40), average values diminished only 8% in one hour, and when activity was below 10, no diminution was observed. No drop in activity occurred during incubation up to 60-90 minutes if 10^{-4} M zinc were present. Full restoration of activity resulted if after saline incubation for 60-90 minutes cells were exposed to 10^{-4} M zinc for 15 minutes prior to testing. Zinc partially protected or partially restored control values in high activity cells even after 24 hours. Greatly diminished values present after storage at 4 C. for several days to 6 weeks were increased several fold by exposure to 10^{-4} M zinc (optimal), while magnesium was ineffective. Incubation in 0.3% EDTA in saline for one hour reduced values 79%. Exposure to 10^{-4} M zinc after washing largely restored initial activity, whereas magnesium was again ineffective. It appears that zinc may play an important role in leukocyte alkaline phosphatase activity and is possibly concerned with the marked lability of this leukocyte enzyme in disease.

A Study of Granulocyte Kinetic Models

By *John W. Athens, Alvin M. Mauer, Homer R. Warner, Helen Ashenbrucker, George E. Cartwright and Maxwell M. Wintrobe*. Salt Lake City.

A method for labelling granulocytes with radioactive diisopropylfluorophosphate (DFP^{32}) has been described previously. In normal subjects a triphasic curve has been obtained. In Phase I there is an exponential fall in radioactivity with a half-life of 7 hours. Phase II is a period of almost constant radioactivity with a mean duration of 10 days. Phase III consists of

a fall in radioactivity with a mean half-life of 3 days.

A number of kinetic models have been subjected to analogue computer analysis. The simplest model applicable consists of a random migration of granulocytes from the circulation into the tissues, from which there is no return. Granulocytes leaving the circulation are replaced by cells from a bone marrow maturation pool. The granulocytes in this marrow pool are incapable of division and leave in a sequential manner according to their maturity. The maturation pool is in turn supplied cells by a mitotic pool. For this model, it is assumed that DFP^{32} injected intravenously labels circulating granulocytes to a high level and granulocytes in the bone marrow to about ⅓ of this level.

Calculations based on this model indicate that the circulating granulocyte mass turns over 2.6 times/day; the maturation pool is 25 times the size of the circulating granulocyte mass; the mitotic pool is 8 times the size of the circulating granulocyte mass. In an average man, 80×10^9 granulocytes leave the circulation each day. The time from the last division to entrance into the circulation is 10 days. The mitotic pool doubling time is about 3 days.

The Granulocytic Activity of Plasma Obtained from Normal and Leukemic Subjects

By *H. R. Bierman, G. J. Marshall, T. Maekawa and K. H. Kelly.* City of Hope Medical Center, Duarte, California.

The intraperitoneal infusion of the plasma of normal subjects induces a transient granulocytosis in Sprague-Dawley and Wistar rats. The granulocyte concentration increases within 3 to 6 hours and decreases to the initial level in 12 hours.

The granulocytic response of rats (6 hours) following the infusion of normal plasma was significantly higher ($P < 0.01$) than that induced by isotonic saline. Further, when the absolute granulocytic concentration of the treated Sprague-Dawley rats (6 hours) was compared to the granulocyte concentration of 84 untreated rats, the granulocyte response was also significant at the $P < 0.001$ level.

Following the stimulation of leukopoiesis in man by leukocyte withdrawal, the potency of this substance was increased from 377 to 1080% at 4 to 6 hours.

Assay of this circulating granulocytic stimulant from 20 leukemic patients before and after treatment indicated close clinical correlation with the clinical status of the disease.

Intrathecal Methotrexate in the Treatment of CNS Complications of Acute Leukemia

By *Carol B. Hyman, Charles A. Brubaker and Phillip Sturgeon.* Division of Hematology, Los Angeles Childrens Hospital, and Department of Pediatrics, University of Southern California School of Medicine.

This reports our experience with methotrexate administered intrathecally to 9 children with CNS complications of acute leukemia. The patients were 2.5-11.5 years of age; they received 3 to 5 doses of 0.2 mg./Kg. during a 6- to 12-day period. Symptoms of CNS involvement were the prime indications for therapy; nevertheless, spinal fluid findings were diagnostic in all but one case.

Symptomatic improvement began in 8 instances 2-10 days after the first injection; in one it began immediately following the diagnostic lumbar puncture. In another patient the progress of an ascending paralysis was stopped, but there was no other symptomatic improvement. A state of partial symptomatic improvement was obtained in 4 instances, and in 4 others the symptomatic remissions were complete. Two of the complete remissions occurred in the same patient; they lasted 125 and 140 days, respectively. The other two complete remissions, in different patients, lasted longer than 78 and 71 days. The duration of the partial remissions was 60, 38, 27 and 8 days. Spinal fluid cell counts in 7 of the cases showed a decrease within 2-6 days after the initial dose and were normal in 6-28 days.

At present, x-ray is the usual therapy for the CNS infiltrations of leukemia. The availability of methotrexate for intrathecal administration provides a new therapy. Our experience suggests that when administered intrathecally, methotrexate is at least as effective as x-ray.

Some Observations on the Treatment of Post-irradiation Hematopoietic Depression in Man by the Infusion of Stored Autogenous Bone Marrow

By *N. B. Kurnick, Bernard H. Feder, James C. Gerdes and Andrew Montano.* Department of Medicine, University of California Medical

Center, Los Angeles, and V. A. Hospital, Long Beach.

It was the aim of these studies to determine the efficacy of autogenous bone marrow in producing repopulation of irradiation-depressed bone marrow.

By slow freezing in glycerol it is possible to preserve nucleated bone marrow cells in viable condition for long periods. We have collected and stored marrow in this manner for a number of patients who were to receive extensive radiotherapy for malignant tumors. In 4 such patients, we have reinfused the diluted bone marrow intravenously following the completion of a course of radiotherapy on 5 occasions. No adverse reactions were noted. One of the 4 patients died of acute radiation injury 6 days after the completion of radiotherapy. In the other 3 individuals, the bone marrow became hyperplastic within 3 weeks after each of the 4 bone marrow infusions. Recovery of the peripheral blood counts resulted within a month to 6 weeks. Three other individuals who showed much less severe bone marrow depression following extensive radiotherapy for seminoma served as controls. The bone marrows of these patients, who did not receive infusion of the stored marrow cells, were still hypoplastic 4 to 9 months after completion of the radiotherapy. The peripheral leukocyte and thrombocyte counts were similarly slow in recovering. The results suggest that bone marrow infusion may result in repopulation of radiation-depressed bone marrow. The approach deserves continued study for patients who are to receive rapid extensive radiation or bone marrow–depressing chemotherapy. The storage of bone marrow of normal individuals who are likely to be exposed to excessive radiation in their occupations may also be indicated.

Vascular Fragility as a Factor in the Hemostatic Defect Produced by Dextran in the Hamster

By *Rajendra G. Desai and George P. Fulton.* Stanford University School of Medicine, San Francisco, and Department of Biology, Boston University, Boston.

In view of the recently described hemostatic defect produced by dextrans in man, various dextran solutions and other plasma expanders have been evaluated for their effects upon vascular fragility and other hemostatic mechanisms by means of the hamster cheek pouch.

Normal hematologic values for the hamster have been determined by routine procedures, and re-studied after intravenous injections of dextrans, polyvinylpyrollidone, etc. Bone marrow and hemostatic studies, viz., coagulation time, bleeding time, prothrombin time, clot retraction and thromboplastin generation tests have been performed. In vivo tests, such as moccasin snake venom, negative pressure and microelectrode tests, were used to detect petechial susceptibility and vascular fragility. In vivo observations were recorded on a 16 mm. color movie by using time lapse, cinephotomicrographic technic.

The immediate effects of infusion of dextran were those of hemodilution and transitory vasoconstriction of arterioles, together with an unexplained tendency of white cells to stick to the endothelial wall. From 24 hours until the 4th day, a hemostatic defect was observed in the form of an increase in bleeding time and vessel wall fragility, as demonstrated by microelectrode tests.

Direct evidence has been presented to show that the bleeding defect caused by dextrans is due to the alteration in the integrity of the vessel wall rather than to a defect in platelets or coagulation factors.

Coagulation Studies of Patients with Functional Uterine Bleeding

By *Arthur J. Seaman and Ralph C. Benson.* Departments of Medicine and Obstetrics and Gynecology, University of Oregon Medical School, Portland, Oregon.

Forty patients with functional uterine bleeding have been examined in the Coagulation Laboratory to determine the incidence of underlying bleeding diathesis. The population examined, while not a truly consecutive series, represented most of the instances in which no demonstrable, local pathologic change was present on pelvic examination.

Test systems employed consisted of: clot retraction, bleeding time, Quick thromboplastin time, asolectin partial thromboplastin time, combined prothrombin-proconvertin activity, proaccelerin activity and (in selected instances) thrombin time, thrombocyte count, quantitative fibrinogen and thromboplastin generation test.

Four patients were found to have an underlying hemorrhagic disorder. One had vascular hemophilia (prolonged bleeding time and antihemophilic globulin deficiency); two more had

pseudohemophilia (prolonged bleeding time). Only one of these had a family history of bleeding. The 4th patient had a complex coagulation defect manifested by prolonged bleeding time, abnormal serum thromboplastin generation and fibrinogenopenia without overt fibrinolysis. Thrombocytopenia was not demonstrable in any of these patients.

The patient with vascular hemophilia gave a history of having undergone uterine dilatation and curettage for bleeding on 3 occasions with life-threatening, post-operative hemorrhage and multiple transfusions required for control. The patient with pseudohemophilia and family history of this disease had also been subjected previously to dilatation and curettage. A prolonged bleeding time was the only common manifestation in the patients with bleeding diatheses, emphasizing the usefulness of this simple procedure. The importance of avoiding elective surgery in patients with hemorrhagic disorders is stressed.

BLOOD PROTEINS

Studies of Plasma Albumin Turnover

By *Sheldon Margen and Harold Tarver.* Department of Biochemistry, University of California School of Medicine, San Francisco.

Among the observations noted by us in the course of investigation of albumin turnover, employing various single and double labeling technics, have been the following:

(1) The most rapid turnover rates are observed when I^{131}-labeled albumin is employed. This rate is much more rapid than that seen with internally, biologically labeled material. (2) A new technic of labeling proteins with amino acids by forming the carbamino anhydride of the amino acid and reacting this with albumin has been developed. Employing this method, albumin labeled with S-35 methionine appeared to give turnover rates comparable to biologically labeled albumin.

In order to clarify these observations, the following further studies have been carried out:

In one series, S-35 cystine and methionine biologically labeled albumin was further labeled with C-14 methionine by means of the carbamino anhydride technic. In one instance this doubly-labeled protein was then iodinated with I^{131} to give a triply-labeled protein. The disappearance rates of the individual isotopes, in recipient patients, were then determined.

In another series, biologically S-35 amino acid–labeled albumin was further labeled with I^{131} diiodotyrosine by the carbamino anhydride technic. Both isotopes were then determined in the recipient animals.

The results demonstrate that the C-14 methionine behaved like the internal biological S-35 label. The I^{131} disappearance rate was rapid. The I^{131} label in the carbamino I^{131} diiodotyrosine protein label behaved like the conventional I^{131}-labeled albumin.

Clotting Defects in the Dysproteinemias and Paraproteinemias

By *Seymour Perry, William A. Skoog and William S. Adams.* Department of Medicine, University of California School of Medicine, Los Angeles.

A hemorrhagic diathesis is a frequent and sometimes serious complication in diseases and syndromes in which an abnormality in plasma proteins is a prominent feature. Twenty patients with various dysproteinemias and paraproteinemias, including 16 with multiple myeloma, have been studied with a series of clotting tests. A wide variety of defects were found: thrombocytopenia in 5 patients, thrombasthenia in 5 patients, prothrombin deficiency in 3, accelerator factor deficiencies in 7 and an AHG defect in one patient. There was one patient with a fibrinolysin and one with a circulating anticoagulant.

In an attempt to correlate the various defects with the plasma protein disturbances, several theories have been advanced in which it is postulated that plasma protein disturbances interfere with hemostasis. With this in mind, it became important to study the effect of a favorable change in the plasma proteins in response to therapy in a patient with multiple myeloma complicated by a hemorrhagic diathesis. This was a 52-year-old white man whose sole presenting complaint was serious and protracted bleeding following a hemorrhoidectomy. Serum proteins were 19.1 Gm.% with 17.8 Gm.% globulin with a sharp peak in the gamma zone on electrophoresis. Clotting studies revealed severe defects involving prothrombin, the accelerator factors and

the platelets, as well as activation of the fibrinolytic mechanism. No circulating anticoagulant was present.

One month following combined therapy with prednisone and testosterone, the serum protein had fallen to 12.6 Gm.% with 11.2 Gm.% globulin, urinary proteins had disappeared, and there were no bleeding manifestations. At this time there was a remarkable change to normal in almost every clotting test and a disappearance of the fibrinolysin.

For the final 7 months of the patient's life, bleeding was a very minor problem until an episode of massive hemorrhage from a rectal vessel occurred late in his course. Clotting studies remained normal, except for the appearance of a qualitative and quantitative platelet defect.

In view of the unusual improvements in this patient's serum proteins concomitant with the improvement in his coagulation, more evidence is obtained for the possible importance of abnormal proteins in the pathogenesis of the bleeding diathesis in diseases characterized by protein disturbances.

Plasmapheresis in a Case of Waldenstrom's Macroglobulinemia

By *William A. Skoog and William S. Adams.* Department of Medicine, University of California School of Medicine, Los Angeles.

Plasmapheresis has been found to be a useful technic in quantitating plasma protein synthesis in health and in disease.

The present study was undertaken to evaluate the results of plasmapheresis in a case of Waldenström's macroglobulinemia with an associated cryoglobulinemia.

The patient was placed on a metabolic balance regimen and maintained on a fixed normal dietary intake throughout the study. Balances of nitrogen, P, Ca, Cl, and Na and K were determined. Urinary protein and uric acid were also measured daily. After a control period of 15 days, the subject was bled 500 cc. per day for 15 days. The plasma was separated by centrifugation and erythrocytes reinfused. A total of 500 Gm. of protein in 5700 cc. of plasma were removed by this technic. A final control period was continued for 10 days.

Plasmapheresis was observed to lower the abnormal plasma protein fraction from 7.5 Gm.% to 4.5 Gm.%, whereas the concentrations of the other plasma proteins remained unchanged. By the last day of the final control period, however, the abnormal protein concentration had risen to preplasmapheresis levels. The patient's pronounced retinal venous dilatation and "sausage" segmentation were less marked at the end of the plasmapheresis period. The negative nitrogen balance during plasmapheresis was rapidly compensated for by diminished urinary nitrogen excretion. At the same time, urinary protein excretion also fell. Na and Cl urinary excretion responded similarly to that of nitrogen. P and K balances were slightly negative during plasmapheresis, reflecting tissue breakdown. Ca balance remained in equilibrium and urinary uric acid levels were unchanged.

This study demonstrates that the elevated plasma paraprotein levels in a case of Waldenström's macroglobulinemia may be lowered by plasmapheresis. Preplasmapheresis levels were rapidly re-established following discontinuance of the procedure.

CARDIOVASCULAR SYSTEM

Differential Coronary Perfusion with Venous and Arterial Blood

By *Russell M. Nelson, John M. Peters, Carl R. Peterson, Dennis W. Christensen, David R. Haymond and C. Gordon Frank.* Department of Surgery, University of Utah College of Medicine, Salt Lake City.

It has been suggested by Beck and others that spontaneous fibrillation occurs when the heart is subjected to differential perfusion of blood of different oxygen saturation. For this reason, this experiment was designed to assess whether or not spontaneous arrhythmias might occur in the dog heart when one coronary artery was perfused with arterialized blood and the other coronary artery perfused with venous blood.

The method of this experiment utilized the isolated dog heart prepared by cannulating each main coronary artery and arranging a system of reservoirs so that each coronary artery could be perfused with either venous or arterial blood. A Sigmamotor pump was employed to propel the blood from a donor dog to the isolated heart.

The blood then returned to the donor dog by a gravity flow. Continuous electrocardiographic patterns were monitored throughout the perfusion.

The results obtained showed that the sudden change from arterial blood to venous blood in either coronary artery was not associated with the occurrence of spontaneous ventricular fibrillation or other arrhythmias. The "pink and blue" heart resulting from this kind of perfusion was not associated with any discernible disturbances of rhythm.

The Nature of Acute ST Changes in in Experimental Coronary Occlusion

By *Werner E. Samson and Allen M. Scher.* Departments of Medicine and Physiology, School of Medicine, University of Washington, Seattle.

Investigations were carried out to determine the nature of the ST segment shift with acute myocardial injury. The following problems were studied: (1) Is there an apparent ST shift due to TQ displacement and/or is there a true ST segment shift? Conventional EKG recorders cannot differentiate these changes. (2) If there is a TQ displacement, is partial depolarization of the injured area the source of the injury current? (3) If there is a true ST segment shift, is it due to an alteration of conduction or due to a change in activity of the individual myocardial cells in the injured region?

Standard lead II electrocardiograms and chest leads were recorded in open-chested dogs through a direct (non-condenser) coupled recorder. Intramyocardial potentials were recorded to determine conduction pathways with multipolar (30 points/3 cm.) electrodes and a multichannel oscilloscope. Intracellular potentials were measured with a Ling-Gerard ultramicroelectrode. Recordings were taken before, during and after temporary ligation of a main coronary arterial branch.

Definite ST shifts with smaller TQ shifts in opposite direction were found within 60 seconds after tightening the ligature. Bipolar intramural complexes obtained within 30 to 120 seconds after ligation often showed changes suggestive of local conduction disturbance. Decrease in excitation velocity, however, was slight. Intracellular records showed a distinct decrease of the resting potential and shortening of the repolarization plateau in the injured area approximately 30 seconds after tightening the ligature.

The usual explanation of the baseline shift is that injury current flows from resting to partially depolarized injured areas, which would produce a TQ shift. Our investigations confirm this. In addition, we found a true ST shift which was not due to regional lack of depolarization during electrical systole as has been theorized but rather to faster repolarization in the injured region.

Reversal of Quinidine-induced Retardation of Myocardial Depolarization by Sodium Lactate and Hyperventilation

By *H. Lenox H. Dick and Elton L. McCawley.* Departments of Medicine and Pharmacology, University of Oregon Medical School, Portland.

Several investigators (Bellet, Wasserman, Dick and McCawley) have shown that sodium lactate can reverse some of the EKG changes of "toxic" doses of quinidine. The purpose of this report is to question whether or not quinidine's toxic widening of the QRS wave may be secondary to other changes.

Molar sodium lactate effectively narrows the QRS, and P waves widened as a result of 80 mg./Kg. quinidine gluconate in dogs. Although there is significant (25%) broadening of these waves following 20 mg./Kg. quinidine, it is unchanged by sodium lactate. With the larger dose of quinidine there is also hypotension, peripheral vasodilation, acidosis and respiratory depression. Sodium lactate reverses these effects of quinidine, suggesting that the alteration of QRS and P wave width may be the result of these presumably nonmyocardial changes.

Dogs given large (60-80 mg./Kg.) doses of quinidine were placed in a tank respirator and minute volume doubled or trebled. The loss of CO_2 with resulting respiratory alkalosis reverses quinidine's broadening of QRS and P waves. Inhalation of 2.5% CO_2 and 97.5% O_2 causing a similar increase in minute volume but with the production of a respiratory acidosis did not, however, alter quinidine's widened QRS or P waves.

Respiratory acidosis or alkalosis by altering the Donnan membrane equilibrium changes the sodium-potassium ratio across cell membranes as well as its electrical potential. Quinidine's retardation of the rate of atrial and ventricular depolarization (width of P and QRS waves) may thus result from cellular shifts of cations due to acidosis.

Observations on Right Bundle Branch Block

By *Morton Lee Pearce.* V. A. Hospital, Los Angeles, and UCLA School of Medicine.

Various categories of patients with RBBB were reviewed in an effort to clarify the problem of the diagnosis of myocardial infarction and ventricular hypertrophy complicating this conduction defect.

The findings are summarized as follows: (1) Intermittent RBBB: The first 0.3-0.4 seconds of ventricular depolarization is not altered by RBBB. (2) Spatial vectorcardiograms. A corrected lead system (Frank) was compared with a tetrahedron (Burch). Equally strong rightward and anteriorly directed terminal forces occurred in each system. (3) Seven patients with RBBB who had no evidence of hypertrophy or infarction at autopsy: One had an R′ V_1 = 1.3 mV. The largest left precordial R was 1.1 mV. (4) Fifteen patients with RBBB and autopsy localization of myocardial infarction: The lesions were correctly localized in 13. (5) Twenty patients with RBBB and autopsy diagnosis of ventricular hyptertrophy without infarction: Only 5 of 13 patients with right ventricular hypertrophy had an R′ V_1 > 1.5 mV. Only 5 of 14 fulfilled the usual criteria for left ventricular hypertrophy.

The following conclusions are suggested: (1) The strong rightward and anterior forces of RBBB seen in precordial leads are probably not due to proximity effects. (2) Since myocardial infarcts are isolated largely to the left ventricle, and early ventricular depolarization is not affected by RBBB, it is not surprising that the electrocardiographic diagnosis of infarction with RBBB is highly accurate. (3) Since strong right ventricular forces are developed after .03-.04 seconds in RBBB, it should be expected that the large S V_1 + R $V_{5\text{-}6}$ of left ventricular hypertrophy would be partially cancelled. Also, these forces must be allowed for before the diagnosis of right ventricular hypertrophy is made.

Lack of Correlation between Myocardial Oxygen Tension and the Electrocardiogram

By *Naci Bor and Peter F. Salisbury.* Intensive Treatment Center and Department of Medical Research, St. Joseph Hospital, Burbank, California.

Contrary to classical belief, the electrocardiogram and tissue oxygen tension are not correlated when both are taken from the same area of the heart muscle. Tissue oxygen tension was measured with open-tip, blank platinum electrodes: this method has become reliable, reproducible and stable in our hands because we have modified the electrodes so as to avoid breaks of the insulation and "poisoning" of the metal surface. Identical measurements were obtained for many weeks with repeated calibrations.

As measured here, the tissue oxygen tension in heart muscle is not a factitious measurement but represents real physiologic entities. It was closely related to the oxygen supply (ml. oxygen supplied to 100 Gm. heart per minute) and also to the "oxygen supply/oxygen consumption ratio." It was not related to other parameters.

S-T segment depressions or elevations were induced in thoracotomized dogs by hypotension. Inhalation of 100% oxygen did not cause reversal of the induced ECG changes even though the oxygen tension of the myocardium increased significantly. In other experiments, a branch of a coronary artery was ligated. Oxygen inhalation increased the oxygen tension in the marginal areas of the ischemic region but did not produce parallel improvements of the local ECG: while the pO_2 rose significantly (even up to 200% of the control value before ischemia), the local electrocardiographic abnormality persisted.

Epicardial application of electrolyte solutions caused marked abnormalities of the local ECG. Inhalation of pure oxygen did not cause any normalization of these, although the myocardial oxygen tension did increase. Similar ECG changes occurred after intravenous infusion of electrolytes: they were reversed by 50% glucose i.v. Myocardial pO_2 remained unchanged during the infusion experiments.

Design of a Clinical Ultra Low-Frequency Ballistocardiograph

By *Robert R. Donaldson, Don M. Cunningham, Herbert E. Griswold and Ralph B. Reaume.* Division of Cardiology, Department of Medicine, University of Oregon Medical School, and Department of Engineering Design, University of California, Berkeley.

Investigation by a number of workers indicated that the original high-frequency and direct-body technics introduce a large degree of distortion in the wave forms of the recorded ballistocardiogram. The development of an ultra low-frequency system has demonstrated a more acceptable record and potentially offers more useful

clinical data than previous ballistocardiograms. A clinically useful platform has been designed which employs horizontal rods to offset the supporting wires from the vertical suspension. This arrangement performs a dual function, lending motion to the longitudinal axis of the body and permitting attainment of ultra low–natural frequencies without the use of excessively long wires. Since platform weight is a critical point, a special honeycomb cellular aluminum core has been utilized. The total weight of the platform, including headrest support and various pick-up transducers, is such as never to exceed a total of 7 lbs.

The natural frequency of this platform oscillation is 0.16 cycles per second with a 75 lb. load increasing the load to 200 lbs. and reducing the natural frequency to 0.11 cycles per second. The platform damping values range from 5 to 10% of the critical for loads between 75 and 200 lbs.

An acceleration recording has been secured by use of Schaevitz model HG-5 accelerometers which are connected to carrier preamplifier units of the Sanborn 150-108 four-channel recorder.

Sample records on patients, both with normal cardiovascular system and abnormalities, have shown that there is great consistency from cycle to cycle.

Relationships Between Stroke Volume, Cardiac Output and "Central Blood Volume" in Cardiac Patients

By *Leonard A. Cobb, Lewis A. Ralston and Robert A. Bruce.* Department of Medicine, University of Washington, Seattle.

Studies designed to describe the pathophysiologic relationships of "central blood volume" (CBV) as determined by the Stewart-Hamilton method were undertaken in patients with left ventricular diseases. Positive correlations between the dependent variables of CBV and cardiac output (CO) have been noted previously in mitral stenosis and in normals (Rappaport, Johnson). The importance of CBV in the regulation of cardiac output has been suggested by others. Indicator dilution curves were obtained by injecting Evans blue dye into the main pulmonary artery. Samples at 2-second intervals were taken from the brachial artery, using a multiple fraction collector.

CBV decreased in all of 6 patients with left ventricular failure who were studied before and after usual medical therapy (−124 to −556 ml.). Although stroke index (SI) rose an average of 5 ml., resting cardiac output did not change significantly. In the same patients the administration of isoproterenol resulted in an average increase of 116 ml. (−34 to +246 ml.) in CBV and a uniform increase in CO and SI. Ten other patients with left ventricular disease, but not in failure, had similar hemodynamic changes with isoproterenol.

The ratio of CO to CBV varied greatly under resting conditions (1.3 to 6.8). Both therapy and isoproterenol resulted in an increase in the "central turnover" (CO/CBV) in all patients.

Relatively small changes in CBV may be detected in a given patient. Either the "central turnover" or the mean transit time would seem to be a more meaningful description of the central circulation than CBV per se.

The Use of Isoproterenol to Simulate the Hemodynamic Responses to Exercise in Cardiac Patients

By *Robert A. Bruce, John H. Morledge, Leonard A. Cobb, Shigeaki Katsura and James E. Dalen.* Department of Medicine, University of Washington, Seattle.

The possibility of simulating the primary cardiovascular responses of hyperkinemia and vasodilatation of exercise was investigated by intravenous isoproterenol. Lockett has isolated an isoproterenol-like substance from mammalian adrenals, Rushmer and West have studied responses in intact dogs, and Dodge and Murdaugh have increased cardiac output with isoproterenol in patients with heart failure. Fourteen patients had valvular heart disease and one each had hypertension, constrictive pericarditis, complete A-V block or idiopathic dilatation of pulmonary artery. Radial and pulmonary arteries were catheterized to determine pressures and flows (Fick principle) directly. Observations were made in the supine posture before and during steady state responses to .02 to 0.4 μg./Kg./min. isoproterenol intravenously. Following recovery, comparative observations were made during steady state of walking on a treadmill.

Heart rate, cardiac index and cardiac work increased with either isoproterenol or exercise, but the rate of change was more rapid with exercise. Exercise increased ventilation, oxygen consumption and A-V oxygen difference, while isoproterenol reduced the latter. Pulmonary arterial

pressure increased with either experiment, but systemic pressure rose only with exercise, due to the vasoconstrictor response to gravity in upright posture. Pulmonary capillary pressure diminished with isoproterenol in patients with left ventricular failure but increased in those with mitral stenosis.

Since isoproterenol simulates many of the primary cardiovascular responses to exercise even in cardiac patients, and eliminates voluntary performance and metabolic loading of the peripheral circulation, it is a useful agent for the hemodynamic study of heart disease.

The Effect of Heart Rate on Cardiac Output and Arterial Pressure in Dogs at Rest and during Exercise

By *Homer R. Warner, Cloyd C. Hofheins and Alan F. Toronto.* Department of Physiology, Latter-Day Saints Hospital, Salt Lake City.

Although it has long been known that the increase in cardiac output that occurs during exercise is associated with an increase in heart rate, quantitative data are not as yet available regarding the exact relationship between these variables. It is the purpose of this study to present data elucidating this relationship.

In the present experiments, permament cannulation of the right renal artery and vein of right nephrectomized mongrel dogs was performed and the cannulae brought through the skin of the flank. This allowed easy access for pressure recording and cardiac output measurements by the dye dilution technic in the unanesthetized dog, at rest or exercising on a treadmill. After control exercise studies, a complete A-V heart block was accomplished according to the technic of Lewis et al. (Surg. Forum 5:96, 1955). Wires from an electrode sutured into the right ventricle were brought out between the scapulae where they could be connected to a stimulator whose frequency was proportional to an input voltage signal. A square or sine wave input voltage was used to produce corresponding changes in heart rate. Arterial pressure and cardiac output were found to be heart rate–dependent in the exercising and unanesthetized resting dog. However, the same dogs under nembutal anesthesia (25 mg./Kg.) exhibited no such dependency with heart rates from 60 to 150 per minute.

It is postulated on the basis of this and other observations that heart rate itself initiates a reflex which influences cardiac output.

Effects of Partial and Total Heart-Lung Bypass on the Heart

By *Peter F. Salisbury and Naci Bor.* St. Joseph Hospital, Burbank, California.

It has often been claimed that partial heart-lung bypass can "rest" the heart and will therefore have a therapeutic effect in acute reversible cardiac failure. Physiologic experiments which have a bearing on this claim have not heretofore been described, aside from the work of Galletti and Salisbury who defined the conditions under which the work of the left ventricle can be decreased for prolonged periods with survival of the experimental animals. In the present experiments the great veins and the pulmonary artery were ligated and the entire venous return was directed into a "venous pump." Beyond the "venous pump": (1) blood could be directed into the pulmonary artery, so that it was arterialized in the animal's lungs and pumped by the animal's left ventricle; (2) it could be directed into a heart-lung machine, where it was arterialized and whence it was injected into a femoral artery by a pump; or (3) appropriate screw clamps permitted distribution of the output of the "venous pump" between the heart-lung machine and the left ventricle in any desired percentile of the total flow. In this system, all blood appearing in the right ventricle was coronary venous blood, permitting accurate measurement of coronary flow, of cardiac oxygen consumption and of cardiac oxygen supply; while the function of the right ventricle was constant (unphysiologic) and the function of the left ventricle was variable over the entire range from zero output to very high outputs, while the systemic pressure was constant.

Conclusions: In normal dog hearts, partial heart-lung bypass does not influence cardiac oxygen consumption unless the bypass carries well over 50% of the total systemic flow (in some instances 99% of the systemic flow must be carried by the bypass in order to decrease cardiac oxygen consumption). The ratio between cardiac oxygen supply and cardiac oxygen consumption is not influenced by partial bypass, unless more than 75% of the total systemic flow is bypassed. Bypass always decreased left atrial pressure when more than 25% of the systemic flow was bypassed; it often increased left atrial pressure when carry-

ing less than 25% of the total flow. Partial bypass would therefore seem indicated as a treatment for pulmonary edema when this is caused by high left atrial pressure. Conclusions cannot be drawn from our experiments concerning the effect of partial bypass on the function and metabolism of hearts with coronary disease.

Studies in Oxygen Consumption During Cardiopulmonary Bypass

By *Albert Starr, Donald M. Pitcairn and Herbert E. Griswold.* Crippled Children's Division, Department of Surgery, and Department of Medicine, University of Oregon Medical School, Portland, Oregon.

The present study is concerned with the relationship between oxygen consumption, blood flow and metabolic acidosis in humans perfused at a rate approximating resting cardiac output.

Fifteen consecutive patients undergoing total cardiopulmonary bypass were subjected to studies of arterial and venous oxygen saturations, oxygen consumption, arterial pH, pCO_2 and whole blood buffer base. The mean flow was 2.7 L./M.^{2} body surface/min. and held constant. Preliminary studies in 57 dogs revealed the advantages of flows in this range.

There was no deviation from normal in arterial pH and whole blood buffer base in 12 patients. Oxygen consumption varied from 50% to 156% of the predicted consumption. Venous oxygen saturations below 70% occurred only when oxygen consumption was 86% or above of the predicted basal consumption. Three patients developed metabolic acidosis under one of three circumstances: (1) arterial unsaturation, (2) excessive muscular activity, (3) prolonged perfusion.

It is concluded that flows of normal resting cardiac output will usually result in no evidence of metabolic acidosis. Oxygen consumption may be above basal consumption during bypass, and under these circumstances a flow of normal resting cardiac output may be inadequate in preventing mild metabolic acidosis. These findings tend to invalidate a rigid method of calculating an optimal flow rate entirely on the basis of predicted oxygen consumption.

Development of Pulmonary Hypertension in Mitral Stenosis

By *Arthur Selzer.* V. A. Hospital, and Department of Medicine, Stanford University School of Medicine, San Francisco.

The association of mitral stenosis with severely elevated pulmonary vascular resistance (PVR) leads to a well-known clinical syndrome in which signs of mitral stenosis may be overshadowed by those of pulmonary hypertension. This report deals with 5 cases in which the unusual opportunity presented itself in observing the development of the syndrome of mitral stenosis with high pulmonary vascular resistance.

The series consisted of 4 male and one female patient. Their ages ranged from 27 to 50 years. Cardiac catheterizations were performed twice in 2 cases and three times in 3, with intervals ranging from 2 to 4 years. Three patients underwent mitral valve surgery, which was successful in 2 of them. Both of these patients later developed restenosis of the valve, coincidentally with which there was an increase in PVR. The 3rd patient developed significant mitral regurgitation after surgery, and, within 2 years, developed severe pulmonary hypertension. Two patients had no intervening operation, but within 2 and 2½ years, respectively, showed marked increase in PVR. In both of these patients, the calculated mitral valve area was the same at the time of normal and at the time of elevated PVR. The range of increases in PVR in serial studies varied from 3 to 10-fold the original value, with the initial reading varying between 60 and 250 dynes/sec./cm.$^{-5}$ and the final readings between 435 and 1060 dynes/sec./cm.$^{-5}$.

In spite of the small size of the series, the following observations are noteworthy: (1) pulmonary hypertension may develop rapidly; (2) it may develop without increase in severity of mitral stenosis; (3) it may be initiated in the 3rd, 4th and 5th decade of life.

It is concluded that high pulmonary vascular resistance cannot be considered, as suggested by some, a "protective" adaptive phenomenon in response to a critical reduction of mitral valvular area but rather appears to be a complication of mitral stenosis, not necessarily related to its severity or duration.

Central Circulatory Changes Accompanying Mitral Commissurotomy

By *Harold G. Richman, J. Bradley Long and Elliot Rapaport.* Department of Medicine, University of California Medical School, and Cardiopulmonary Laboratory, Mount Zion Hospital, San Francisco.

Previous studies on the immediate circulatory effects of mitral commissurotomy have been limited to mitral valve pressure gradient measurements and have neglected the effect of mitral valve flow. This study was undertaken to evaluate the immediate effects of commissurotomy on pressure-flow relationships at the mitral valve and in the pulmonary circulation.

Left atrial, left ventricular, pulmonary artery and brachial artery pressures were obtained in 18 patients during stable periods immediately before and after commissurotomy. Simultaneous dye dilution cardiac outputs were determined using left atrial injection of T-1824.

Preoperative left atrial diastolic pressure averaged 19 mm. Hg (range 13 to 30) and fell to a mean of 11 mm. Hg (range 7 to 16) following fracture. This was associated with a decrease in mitral valve diastolic gradient averaging 11 mm. Hg (range 5 to 19). Mean precommissurotomy cardiac index was 2.7 L./min./M.^{2} (S.D. .54). The ratio of cardiac index before to after commissurotomy averaged 1.05 (S.D. .15). Injection to sampling site volume ranged between 405 and 1150 cc., with no consistent change after valve fracture. Pulmonary arteriolar resistance varied from 80 to 1330 dynes/sec./cm.$^{-5}$. Following commissurotomy, mean pulmonary artery pressure fell proportionately to the decrease in left atrial pressure. Consequently, the ratio of preoperative to postoperative pulmonary arteriolar resistance remained essentially unchanged (mean = .95, S. D. .27).

In conclusion, mitral commissurotomy results in an immediate fall in left atrial pressure without significant change in cardiac output. The decrease in pulmonary artery pressure which consistently occurs is a passive effect unaccompanied by immediate significant change in the pulmonary vascular resistance.

Evaluation of a Flap Valve Prosthesis in Septal Defect Repair

By *Albert Starr*. Crippled Children's Divison, Department of Surgery, University of Oregon Medical School, Portland, Oregon.

The present study was undertaken to evaluate a flap valve Ivalon sponge prosthesis as a possible means of increasing the safety of closure of septal defects with bidirectional shunting.

Fifteen dogs were subjected to cardiopulmonary bypass and the entire atrial septum excised. The defect was closed with a double layer compressed Ivalon patch, one layer having an 8 mm. hole and the other acting as a flap valve hinged at one end.

Thirteen dogs survived the procedure, and function of the flap was studied by cardiac catheterization and angiocardiography using acetylcholine arrest. Dogs were sacrificed at varying intervals and pathologic examination carried out to determine the mechanism of healing.

Satisfactory closure of the defect occurred in all dogs sacrificed more than 4 weeks following insertion, and patency was demonstrated in most dogs sacrificed earlier. An 8 mm. atrial septal defect cannot be detected by cardiac catheterization but can be easily demonstrated by angiocardiography using cardiac arrest.

This study provides an experimental basis for the treatment of patients with septal defects, pulmonary hypertension and bidirectional shunting in which left to right shunting can be eliminated at the time of surgery and right to left shunting gradually eliminated at a later date without further operation.

Prediction of Metered Left Ventricular Regurgitation from Multiple Indicator Dilution Curves in Dogs

By *Ramon L. Lange, Hiroshi Kuida and Hans H. Hecht*. Department of Medicine, University of Utah College of Medicine, Salt Lake City.

There is close similarity of dye curves obtained simultaneously from the pulmonary artery (PA) and femoral artery (FA) following venous injection in normal subjects. In mitral or aortic insufficiency, the FA curve is altered when compared with the PA curve. This technic allows some quantitation of valvular regurgitation based on the premise that in valvular regurgitation net forward flow (Q_F) is the resultant of total forward (Q_T) and regurgitant (Q_R) flow. Q_T and Q_F may be represented by (a) the most rapid, and (b) the average traversal of a volume between sampling sites. Comparing differences between the appearance times and mean circulation times of the 2 curves yielded a ratio Q_R/Q_T and a measure of Q_R. The calculations agreed with estimates made at surgery and received support from internal checks. Since precise measurements of regurgitation in man is not possible, the procedure could not be checked against independent observations. Experimental studies in animals seemed necessary.

Simultaneous dilution curves were obtained

from the PA and FA in 10 dogs before and after left ventricular regurgitation was induced by placing an orifice meter between the subclavian or brachiocephalic artery and left atrium. The induced Q_R was obtained from the orifice pressure drop, and Q_F from dye output. The calculated "regurgitation" from PA and FA dye curves agreed well with the measured actual "regurgitation" in ranges of 20% to 70%. The studies validate previously reported dye estimates of regurgitation in human subjects.

Induced Cardiac Arrest as an Aid to Angiocardiography

By *John C. English, William S. Hoskinson, Louis H. Frische and Charles T. Dotter.* Department of Radiology, University of Oregon Medical School, Portland, Oregon.

The induction of cardiac arrest in the closed chest dog by use of the cardioplegic agents acetylcholine or methacholine lowered the pressure gradient between the left and right atrium sufficiently to enable synthetic interatrial septal defects to be visualized during angiocardiography. Injection of the cardioplegic agents via intra-aortic catheter was found to be unnecessary since rapid intravenous injection sufficed to bring about arrest. The induction of arrest of 6 to 8 seconds' duration in more than 50 normal dogs was shown to be without permanent injurious effects. Acetylcholine was found to be superior to methacholine as a cardioplegic agent for angiocardiography because of its more rapid destruction. Measurement of the pressure gradient between the right and left ventricles and between the aorta and pulmonary artery during arrest indicated that visualization of interventricular septal defects and aortico-pulmonary shunts will also be made possible by this technic.

Effects of Clinical Digitalis Levels on Rubidium Uptake of Red Blood Cells

By *Jerold M. Lowenstein, William L. Caldwell and Grover Liese.* Departments of Medicine and Radiology, Stanford University School of Medicine, San Francisco.

Cardiac glycosides in low concentrations inhibit the uptake of potassium and rubidium by red blood cells in vitro. The rubidium uptake of patients' red cells was measured. Washed red cells were incubated at 37° with 2 solutions; (1) potassium-free Ringer's solution with a trace of radioactive rubidium (Rb^{86}), (2) the aforementioned solution plus 5×10^{-6} Gm./cc. digoxin, enough to cause maximum inhibition of rubidium uptake.

When patients receive digitalis initially there is some diminution of uptake (1) and no change in uptake (2). After a variable period of time, uptake (1) returns to normal and uptake (2) is reduced. Unless both phases take place, the patient does not seem to benefit by taking digitalis. Individuals in digitalis toxicity have extreme depression either of uptake (1) or uptake (2). Patients in congestive heart failure and not taking digitalis generally have a low uptake (2), suggesting the presence of a digitalis-like substance in their circulation.

Effect of Arterial Transmural Pressure on Pulse Velocity

By *Chester Hyman and Travis Winsor.* Departments of Physiology and Medicine, University of Southern California, School of Medicine, and Heart Research Foundation, Los Angeles.

A simple, rapid method for the measurement of pulse velocity has been developed. If the "earlier" pulse wave is put onto the X plates of a cathode ray oscillograph, and the "later" pulse on the Y plates, the oscillograph traces an open loop with a shape characteristic of the segment under study. The time required for the tracing to move from its lowest point to the point furthest to the left is a measure of the pulse transit time between 2 detectors. With an interrupted cathode beam, this time may be measured to 0.005 seconds.

Pulse velocity measured between the groin and the ankle in healthy young men can be markedly diminished by the interposition of a cuff inflated to sub-systolic pressures between the two detectors. The delay is detectable at pressures as low as 40 mm. Hg and increases with increases of cuff pressure to mean arterial pressure. The delay at any given pressure can be doubled by interposing 2 cuffs between the detectors.

The velocity can be accelerated by placing the extremity in a closed, partially evacuated chamber.

All the modifications of pulse velocity described are consistent with the hypothesis that a decrease in transmural pressure in an artery slows the passage of the pulse wave, while an increase in transmural pressure increases pulse velocity.

Vectorplethysmography in Health and Disease

By *Travis Winsor and Chester Hyman.* Departments of Medicine and Physiology, University of Southern California, School of Medicine, and Heart Research Foundation, Los Angeles.

Vectorplethysmography is a method for comparing volume changes of pulse waves from 2 parts of the body. The method makes possible accurate analyses of differences in amplitude, time of onset and configuration of the pulsations. The technic is suitable for measuring pulse velocity in various segments of the vascular tree. In normal, young adults the time of onset and configuration of the pulses at various points on the right arm or leg are similar to those at like points on the left arm or leg, as shown by a vectorplethysmogram which runs at a 45° angle with a horizontal reference line. With arteriosclerosis obliterans of mild degree the angular position changes, indicating amplitude differences. With moderately advanced disease an open loop is formed, indicating differences in configuration. With far-advanced disease with a complete or nearly complete obstruction time differences become apparent as the pulse velocity is slowed. With the usual conventions used in our laboratory, when arterial disease involves the limb vessels on the left the loop rotates in a clockwise direction. When disease is present on the right it rotates in a counterclockwise direction. It is of particular interest that multiple lesions in a limb may be located, a factor which assists in the selection of patients for aortic graft or thromboendarterectomy. Normally in the young adult, the pulse velocity in the legs and aortoiliac segment is slower than in the arms. In many elderly patients the velocities are approximately equal. With Monckeberg's arteriosclerosis these velocities exceed those of the arm. With diffuse obliterative arteriosclerosis of the legs the leg velocities are slowed with respect to the arms.

A Study of the Slow (Alpha) Waves of the Plethysmogram

By *Elizabeth M. Cuthbertson, Rutherford S. Gilfillan and Mikel Duino.* Experimental Surgery, Children's Hospital, and Department of Surgery, Stanford University School of Medicine, San Francisco.

Of the 5 waves described in the normal plethysmogram, only the pulse and respiratory waves have been much investigated. Little is known about the slow waves. Many authors state that alpha waves are absent after sympathectomy but give no data or references. Neuman et al. report their presence during spinal anesthesia. Their source, significance and method of control are unknown.

In the present study the method of digital plethysmography was adapted for use on the dog's paw. It has been established that alpha waves are present in the dog's hind paw after denervation by high section of the sciatic and femoral nerves and transection and re-anastomosis of the femoral artery. Sudden marked vasoconstriction or vasodilatation usually abolishes alpha waves, but if the same change occurs gradually (or time is allowed for adaptation to the new state) alpha waves may remain (or return). Alpha waves may be present under anesthesia but may disappear with changes in depth of anesthesia. These waves are usually synchronous in both hind paws, but not always. They may be absent for no apparent reason in a dog which has previously exhibited large alpha waves. Denervation of the carotid and aortic chemoreceptor and baroreceptor mechanisms does not abolish alpha waves. Sympathectomy and sympathetic ganglionic block (TEA) do not abolish alpha waves.

Recent Developments in the Electromagnetic Flowmeter for Measuring Regional Blood Flow

By *G. H. Herrold, E. A. Abbott and R. B. Wilk.* UCLA Medical Center.

The electromagnetic flowmeter for measuring regional blood flow has been improved in its recording circuitry and made into a versatile instrument suitable for human as well as animal research. Provision has been made for the simultaneous recording of pulsatile and mean flow rates. The accuracy of measurement is within 5%.

A ring modulator circuit is used as a phase sensitive detector. The main advantages of this type of detector are excellent linearity and inherent insensitivity to artifacts differing in phase from the desired flow signal.

A differential circuit has been developed to integrate directly the output of the phase sensitive detector. The mean flow circuit integrates positive as well as negative flow and is also insensitive to artifacts differing in phase from the desired signal.

The need for a specially constructed low impedance device has been eliminated, and a circuit designed to accept the high impedance output of a conventional resistance capacitance network has been devised.

In order to avoid the necessity of recalibrating the instrument for each measurement, a grounded annular ring is located at each end of the flowmeter sleeve. This device establishes an essentially constant impedance reference point to ground to the balanced input of the signal amplifier.

An adjustable sensing unit which permits measurement of blood flow from vessels of varying sizes has been developed, thereby eliminating the need for an excessive number of flowmeter units.

Variation in Serum Lipids during Mental and Emotional Stress

By *P. T. Wertlake, A. A. Wilcox, M. I. Haley and J. E. Peterson.* Departments of Biochemistry and Medicine, School of Medicine, College of Medical Evangelists, Loma Linda, California.

A previous study reported by this laboratory described the variation of serum cholesterol in a group of 44 medical students during a control period and in conjunction with the mental and emotional stress of final examinations.

This study has been extended so that 39 of the original group of 44 subjects have now been observed for a 2nd year. Total and esterified cholesterol, phospholipids, alpha and beta lipoproteins have been measured during intervals in which the subjects were relatively free of stress, as well as during the week of final examinations. Psychological evaluation before and after examination week has been attempted for the purpose of assessing the individual response to a stressful situation of this sort.

Each year the mean level of serum cholesterol for this group of subjects has increased significantly ($P < 0.001$) during the week of final examinations. The increase has been due chiefly to a change in the esterified fraction. Changes in phospholipid, alpha and beta lipoprotein, have been noted but are less consistent. Total serum cholesterol has seemed remarkably labile in certain subjects, and this has been of particular interest. We are not able as yet to relate this lability to any particular aspect or severity of stress. The ingestion of safflower oil during a subsequent period of examination stress has reduced the levels but not the degree of change in serum cholesterol.

Effect of Stress on Rats Fed a High Fat Diet in the Development of Atherosclerosis

By *Herman N. Uhley and Meyer Friedman.* Harold Brunn Institute, Mount Zion Hospital, San Francisco.

Recently, it was found in this laboratory that men exhibiting a specific behavior pattern, occurring in association with chronic exposure to severe occupational stress, had much higher serum cholesterols, more rapid blood-clotting times and markedly higher incidence of arcus senilis and clinical coronary disease than did age-paired control groups exhibiting a contrasting overt behavior pattern but having identical diets and patterns of physical activity. It was therefore considered important to study possible effects of stress on these parameters in animals.

A study was conducted in which a series of rats fed a high cholesterol diet were daily subjected for 10 months to repetitive electrical stimulation and compared to controls fed the same diet. At the end of the experimental interval, a comparable moderate hypercholesteremia was observed in the 2 groups of rats, but coronary arteriosclerotic changes were not present in either group.

Therefore, a second series of rats again was fed the high cholesterol diet, but now containing added butter fat. Half of the rats again were daily subjected to another form of repetitive electrical stimulation which, in contrast to the first series of stressed rats, obviously induced marked behavioral changes. At the end of 10 months, marked hypercholesteremia was present, of comparable magnitude in the experimental and control rats. However, the stressed rats exhibited significantly faster clotting times and a 3-fold higher incidence of coronary arteriosclerotic changes than the nonstressed controls fed the same diet.

The Distribution of Triolein I^{131} in the Atherosclerotic Infiltration of Rabbit Aortas

By *Sanford Oscar Byers, Meyer Friedman, Leland Felton and Paxton Cady.* Harold Brunn Institute, Mount Zion Hospital and Medical Center.

Though the ability of the atherosclerotic artery to participate in the general day-to-day metabolism of cholesterol and of phospholipid is now recognized, the triglyceride dynamics of this tissue are not known. The present report describes the participation of atherosclerotic aorta and other tissues in the disposition of orally administered triglyceride I^{131}. Atherosclerotic normocholesteremic rabbits were fed 2.0 ml. of triolein containing 0.73 mc. of I^{131}, or else equal radioactivity as NaI^{131}, on each of 3 successive

days. All rabbits were protected against thyroid absorption of I^{131} by previous administration of KI. Seven days after the initial I^{131} administration the animals were sacrificed and tissue radioactivity compared with that of similarly treated normal nonatherosclerotic controls. Chromatographic analysis of serum samples from triolein I^{131}–fed animals showed all the circulating I^{131} to be bound to triolein. Radioactivity from triolein I^{131} was found in the atherosclerotic aorta, concentrated preferentially in the atherosclerotic infiltration itself. This distribution was revealed clearly by radioautography of aortic segments wherein darkening of the film corresponds to areas of sudanophilic atherosclerotic plaques with almost no radioactivity from normal aortic intima lying between these plaques. Radioactivity also was found in other fat–containing tissues such as adrenal cortex, aortic and perirenal fat. In contrast, little activity was found in "non-fatty" tissues such as adrenal medulla, liver, kidney and thyroid. In normal rabbits no radioactivity was found in the aorta after triolein I^{131}. After NaI^{131} relatively little radioactivity was present in any tissue tested.

A Genetic Basis for Congenital Heart Disease in the Rat

By *Sidney S. Sobin and J. Bennet Olson.* Los Angeles.

We have demonstrated previously that: (1) congenital cardiac defects occur spontaneously and consistently in the white rat; (2) the cardiac abnormalities are similar to those found in man; and (3) the occurrence of such defects can be markedly potentiated by various procedures and/or agents applied to the pregnant rat at a critical time of fetal cardiac development.

Since spontaneous congenital cardiac defects were not previously found in the rat, it became necessary to consider the effect of strain differences in their occurrence. These are the first studies to compare the incidence of spontaneously occurring congenital cardiac anomalies in 3 different strains of rats reared under identical laboratory conditions. One strain was obtained from 2 sources, thus providing 4 groups for study. The micro-macro methods of dissection of new-born rat pups have been fully described.

External defects were rare in all groups and did not indicate the presence of cardiac defects. The rates at which defects occurred in the 4 groups were strikingly different: Wistar (previously reported) 5.2%; Wistar (this study) 5.8%; Long-Evans (I) 24%; Long-Evans (II) 9.8%; Sprague-Dawley 42%.

These studies demonstrate clearly that the incidence of spontaneous congenital cardiac anomalies in the rat may vary markedly in different strains as well as in the same strain originating from different sources. We believe that there is a genetic basis for these differences.

ECOLOGY

Experimental Study of Prognosis

By *Thomas H. Holmes, Joy Joffe, Janet Wright and Thomas F. Sheehy.* Department of Psychiatry, University of Washington School of Medicine, and Firland Sanatorium, Seattle.

The Berle Index (J.A.M.A. *149*:1624, 1952), an instrument for making quantitative estimates of psychological and social assets, was used as a basis for predicting response to treatment of patients with tuberculosis. It was predicted that patients with low scores (few assets) would have the greatest probability of treatment failure. Interview data on 41 patients (16 females, 25 males) admitted to the tuberculosis hospital in 1953 formed the basis for the Berle scores. In 1958, 5 years after admission, the patients were re-evaluated to test the prediction. The results were then compared with those of a 5-year follow-up study of 669 patients discharged after treatment for tuberculosis.

The original Berle scores were divided into low, medium and high categories and were correlated with response to treatment and current psycho-social adjustment. All treatment failures occurred in the low Berle score group. There was a high correlation of alcoholism, male sex, nontuberculous illness and irregular hospital discharge with low Berle scores. Routine follow-up studies on the 669 discharged tuberculosis patients showed these same factors to be prevalent in the group whose disease had relapsed. There was a highly significant incidence of disintegrated psycho-social adjustment in patients with low Berle scores. Conversely, high Berle scores correlated with female sex, satisfactory psycho-social adjustment, and lack of secondary illness, alcoholism or irregular hospital discharge.

There was a striking validation of the hypothesis that the quality and quantity of psycho-social assets provide the basis for estimating prognosis.

ENDOCRINES AND METABOLISM

Metabolic Changes Produced by Human Growth Hormone (Li) in a Pituitary Dwarf

By *Roberto F. Escamilla, John J. Hutchings, William C. Deamer and Choh Hao Li.* Department of Medicine, Pediatrics and Hormone Research Laboratory, University of California, San Francisco and Berkeley.

Human growth hormone prepared by the Li method from pituitaries obtained at autopsy has been administered to a female pituitary dwarf, aged 11 yrs. 5 mos.

Metabolic balances were studied during an initial control period of 17 days and also during periods of administration of the hormone for 5 to 10 days (dosage 5, 10 and 2.5 mg. daily, subcutaneously), with control periods of 7 and 5 days between. Nitrogen retention occurred with all 3 dosage levels but was found to be most effective at the 5 mg. dosage. Retention of sodium potassium and phosphorus also was demonstrated, and there was a slight increase in serum alkaline phosphatase. Calcium excretion was increased initially, but later observations following long-term administration of the growth hormone revealed a shift to calcium retention. The fasting blood sugar level was not affected, although the glucose tolerance curve became higher.

Studies of long-term administration of this growth hormone with particular reference to its effect on height are in progress.

Metabolic Changes Produced by Chymotrypsin Digests of Bovine Somatotropin in Man

By *Vincent C. DiRaimondo, Ernest Gold, Stanley Newman, Rex Bigler, Felix O. Kolb, Choh Hao Li and Peter H. Forsham.* Metabolic Unit, Department of Medicine, University of California School of Medicine, San Francisco, and Hormone Research Laboratory, Berkeley, California.

By partially digesting bovine somatotropin with chymotrypsin it would appear that Li et al. have converted an inactive hormone into an active hormone in man. This STH core was given intramuscularly in doses of 10 and 20 mg. per day to 6 human subjects for periods ranging from 7 to 14 days. Nitrogen retentions ranging from 1 to 5 Gm./day were observed. No sustained retention of sodium or diabetogenic effect were noted. In some instances it was possible to demonstrate a retention of phosphorus and potassium. A patient with disseminated lupus erythematosis while on 20 mg. of prednisolone retained as much as 3 Gm. of nitrogen per day while receiving 20 mg. of STH core. Improved wound healing was noted. Further studies are in progress. Perhaps bovine STH core will prove to be the anabolic agent free of androgenic effects which clinicians have longed sought for.

Preliminary Observations on Prolactin Activity in Human Blood

By *Benjamin Simkin and David Goodart.* Institute for Medical Research, Cedars of Lebanon Hospital, Los Angeles.

Bahn and Bates recently stated that there was no unequivocal demonstration of the presence of measurable amounts of pituitary prolactin in human serum or urine. It is our purpose to present evidence showing the presence of prolactin activity in human blood. Acid acetone extracts of 10 ml. aliquots of whole blood were obtained using the Sulman method for the extraction of chromatophorotropic activity of human blood. These extracts were assayed by a single injection micro pigeon crop weight technic previously described by us, using increase in crop sac weight as the end-point. Results were as follows: (1) The crop weight stimulating activity of single local injections of blood extracts (equivalent to 2.5 ml. whole blood) of 13 lactating women exceeded the activity of 0.05 I.U. prolactin, whereas blood extracts of 6 children had no activity. (2) The intramuscular systemic injection of pooled extracts of lactating women (equivalent to 20 ml. whole blood) resulted in crop weight increases equivalent to that obtained with 8 I.U. prolactin, whereas pooled extracts from children caused no increase in crop weight after similar systemic administration. (3) Recovery of 0.05 I.U. prolactin added to whole blood of children and extracted by the Sulman method was 100% or more in 3 separate experiments. (4) Blood extracts of intact female adult rats injected locally induced an increase in crop weight of 50 ± 4.5 mg., whereas extracts of hypophysectomized rats caused no increase in crop weight, -8 ± 7.5 mg. (5) Prolactin activity present in the blood of an oophorectomized human female disappeared 2 weeks after surgical hypophysectomy. (6) No prolactin activity was found in the blood of 6 normal young men or 7 young women during the first half of the menstrual cycle,

but prolactin activity equivalent to 0.05 I.U. prolactin per 1.5 ml. blood was found in the blood of 10 women during the second half of the menstrual cycle.

Unreliability of Tests of Phosphate Metabolism in the Diagnosis of Hyperparathyroidism

By *Telfer B. Reynolds, Hilda Lanman and Natalia Tupikova.* Los Angeles County Hospital, Los Angeles, and Department of Medicine, University of Southern California School of Medicine.

During a one-year period, 2 groups of patients were studied by analyses of 24-hour urinary Ca, serum Ca, serum phosphate (P), 4-hour phosphate clearance (Cl_p) and % tubular reabsorption of phosphate (TRP). Group A consisted of normal medical students and patients with renal calculi who were believed not to have hyperparathyroidism because of consistently normal serum Ca and P levels. Group B consisted of 10 patients with surgically verified hyperparathyroidism.

In Group B, TRP ranged from 53% to 87%, being above 75% in 5 cases. Only 1 of these 5 had elevated serum creatinine levels. TRP in Group A ranged from 71% to 95% (mean 86%) and was below 80% in 7 cases.

Cl_p in Group B ranged from 11 to 22 cc./min. (mean 17 cc./min.) and in Group A ranged from 4 to 23 cc./min. (mean 13 cc./min.). Ten of the values in Group A were 15 cc./min. or higher.

Serum P was below 3 mg.% in all but 2 patients of Group B. One of these had elevated serum creatinine and the other did not. Serum P was not below 3 mg.% in any subject in Group A.

Twenty-four hour urinary Ca ranged from 88 to 448 mg. in Group A and from 36 to 472 mg. in Group B. One patient in Group B had levels consistently below 50 mg.

The highest serum Ca in Group A was 11.2 mg.%, while in Group B serum Ca ranged from 11.9 to 15.4 mg.%.

Maximal tubular reabsorption of phosphate during an intravenous phosphate load (TmP) was measured in 5 patients in Group B and ranged from 72 to 150 uM./min. Results of this test were often difficult to interpret and sometimes failed to change in the expected direction postoperatively.

In the 10 patients with hyperparathyroidism, serum Ca was the only consistently abnormal test.

The Effect of Parathyroidectomy in the Dog on Serum and Urine Magnesium Levels

By *Helen Eastman Martin, William P. Mikkelsen and Ruth Jones.*

The purpose of this study was to determine if parathyroidectomy in the dog altered the serum and urinary magnesium levels.

Serum and urinary magnesium and calcium levels were determined before and after parathyroidectomy-thyroidectomy in the dog.

Preliminary studies have demonstrated an abrupt fall in both serum calcium and magnesium levels associated with diuresis of these ions. These changes occurred within 3 days of parathyroidectomy. With low serum levels of calcium and magnesium the urinary excretion of these ions diminished markedly. The serum calcium remained low unless large doses of oral calcium lactate and dihydrotachysterol were administered. The serum magnesium, however, after the initial fall, returned within a few days to a normal level.

These findings suggest that the fall in serum magnesium may be related to magnesium diuresis, at the time of calcium diuresis. The mechanisms responsible for the return of the serum magnesium to normal after the initial fall while the serum calcium remained low have not been elucidated.

The Metabolic Fate of I^{131}-Labelled Thyroxine and 3:5:3′ Triiodothyronine in Man

By *Walter L. Arons and Robert O. Gorson.* Hospital of the University of Pennyslvania, Philadelphia.

Plasma and urinary metabolites of I^{131}-labelled thyroxine and 3:5:3′ triiodothronine were determined after the intravenous administration of 200–1400 μc. of these hormones to separate groups of 5 euthyroid subjects each. Plasma and urine samples were collected at intervals of 1, 3, 5, 24 and 48 hours and their butanol extracts subjected to radio-paper-chromatographic analysis in both n-butanol (2N NH_4OH) and tertiary amyl alcohol (2N NH_4OH) solvent systems. Following the administration of labelled thyroxine, radioactive 3:5:3′ triiodothyronine was found in small amounts at 1 to 5 hours in the plasma of 3 of the 5 patients. No other plasma activity except that localized to the thyroxine and iodide areas was consistently found. Six to 17% of the

radioactivity of the injected thyroxine was excreted in 48 hours, mainly in the form of iodide with small amounts of radiothyroxine. Following the administration of radiotriiodothyronine to five subjects, radioiodide alone could be detected in plasma as a metabolic product. Twenty to 33% of the radioactivity of the injected triiodothyronine was excreted in 48 hours, mostly as iodide with small amounts of unchanged triiodothyronine. In 2 of the latter 5 subjects, however, thyroxine activity was also found in the urine samples. Monoiodotyrosine, diiodotyrosine, the acetic acid derivatives of thyroxine and triiodothyronine, 3:3′ diiodothyronine, and 3:3′5′ triiodothyronine could not be found as metabolites in these 10 studies. These data suggest that thyroxine is to a small extent metabolized to 3:5:3′ triiodothyronine in man and that administered triiodothyronine may be converted to thyroxine during renal excretion.

Recovery of Thyroid Function in the Rat After Withdrawal of Propylthiouracil

By *W. P. VanderLaan and Georgiana Jagiello.* Scripps Clinic and Research Foundation, La Jolla, California.

The purpose of this study was to observe the sequence in which measures of thyroid function returned to the normal range upon the withdrawal of propylthiouracil after its prolonged administration. Plasma protein-bound iodine, thyroid weight and iodine concentration, and thyroid uptake of I^{131} per 5 hours were used as indicators of recovery. After propylthiouracil was withdrawn, 4 rats were killed per day for 5 days, less frequently thereafter.

With high iodine content diet the plasma protein-bound iodine returned to normal 48 hours after propylthiouracil was withdrawn, thyroid size slowly regressed and iodine stores reaccumulated over an 11-day period. Ninety-six hours after withdrawal, I^{131} uptake was 19% per 5 hours, well above the values at 72 and 120 hours.

With low iodine diet the recovery from hypothyroidism was delayed; at 96 hours protein-bound iodine was normal and I^{131} uptake 43%. Thyroid size regressed to ⅛ the original value over 14 days, although I^{131} uptake remained about 30%. Iodine repletion in the gland was slight.

It was concluded that plasma protein-bound iodine is most quickly restored in the recovery from this type of hypothyroidism; the rates of repletion of iodine content of the thyroid gland and regression in thyroid size depend on the iodine content of the diet.

Studies of a Highly Sensitive Method of Insulin Bioassay

By *Paul M. Beigelman.* University of Southern California Medical School, and Los Angeles County Hospital, Los Angeles.

A method of assay for insulin and insulin-like substances has been devised, utilizing glucose uptake by Wistar rat epididymal adipose tissue. Segments of tissue are incubated in Krebs-bicarbonate buffer, containing glucose, in which varying concentrations of insulin are incorporated. A Dubnoff Metabolic Shaker is employed, and the incubation proceeds for 2–4 hours, at 36.5 C., under 95% O_2–5% CO_2. Various factors possibly influencing this bioassay have been examined, including length of incubation time, weight of adipose tissue, initial glucose concentration, nutritional status of the whole animal, effect of varying concentrations of protein in the medium and weight of the animal. The most important factor appears to be the weight of the animal. Tissue from animals weighing 100–180 Gm. are highly responsive to small concentrations of insulin, with a highly significant coefficient correlation (r) of 0.54 evident in the 1–100 microunit/ml. range ($P<0.001$). The sensitivity of the assay diminishes as the weight of the animal is increased. The r value associated with animals weighing over 350 Gm. is 0.22 ($P>0.4$). At present, adipose tissue segments weighing 40–60 mg. are utilized from animals in the 100–180 Gm. weight range, initial total glucose is 2.0–3.0 mg. per beaker, and incubation proceeds for 2–3 hours.

Clinical studies of normal individuals and of diabetic coma have indicated the clinical value of this procedure. Serum protein fractions, obtained by preparative electrophoresis with the Spinco C-P continuous flow paper electrophoresis apparatus, have been tested in this system. Beta-globulin and adjacent fractions appear to have the maximum insulin-like activity.

Effects of Oral Antidiabetic Agents on the Automatism of the Isolated Heart

By *David Jensen, Thomas H. Lambert and Arne N. Wick.* Scripps Institution of Oceanography, University of California, and Scripps Clinic

and Research Foundation, La Jolla, California.

This investigation was designed to ascertain if certain oral antidiabetic agents exerted an effect on the isolated, spontaneously beating heart. Young adult, female rabbits were sacrificed and the heart rapidly extirpated. Threads were sutured into the auricular appendages, and the ventricle removed. The joined beating auricles were then suspended in a chamber containing a suitable medium at 30 C. and gassed with 95% O_2–5% CO_2. A strain gage coupled to an oscillograph recorded rate and force of the beat, while electrodes on each auricle permitted simultaneous and continuous recording of the electrocardiograph on the other channel of the oscillograph. The following drugs were tested: phenethylbiguanide, tolbutamide, carbutamide, chlorpropamide and tolhexamide. The latter 3 compounds, practically insoluble in aqueous media, were administered to the test heart as a fine suspension. A dose of 1 mg. of drug per ml. of medium was chosen. Other dosage levels are being studied at present. The pH of the drug solutions approximated that of the medium. Bizarre changes occurred in the EKG with each of the compounds tested. These changes were accompanied by marked arrhythmias such as fibrillation, although individual susceptibility of a given auricular preparation must be considered in the interpretation of these results. These preliminary observations indicate that any one of these 5 hypoglycemic compounds is capable of inducing derangement in activity of the rabbit heart under the conditions used in this study.

Preliminary Experiences with Chlorpropamide in Diabetic Patients

By *Thomas H. Lambert*. Scripps Clinic and Research Foundation, La Jolla, California.

This report is an early clinical evaluation of chlorpropamide, a new sulfonylurea derivative and orally active antidiabetic agent. Thirty patients with known diabetes of the adult stable, brittle or juvenile type have been studied 5 days to 7 months following administration of chlorpropamide. They were maintained on a restricted diabetic diet prior to chlorpropamide therapy, had insulin withdrawn or had a placebo substituted for chlorpropamide during the period of observation. Capillary blood glucose (true) determinations by the Semogyi-Nelson method were done fasting and 3 hours following breakfast and lunch. Twenty-four-hour excretion of glucose in the urine was measured for each patient, and urine glucose determined 4 times daily by the Benedict method. Kidney, liver-function and hematologic studies have revealed no toxic effects to date. Annoying side effects of nausea, headache, drowsiness, muscular weakness and dizziness, that occurred early in the study with dosages of one gram or more daily, were seldom present with dosages less than 750 mg./day. Seven patients responded with excellent control, 6 with good control, and 2 with poor control. There was no evidence of blood sugar lowering effect in 15 patients. Preliminary observations indicate that chlorpropamide, a more potent hypoglycemic compound than tolbutamide and with a longer duration of action, may prove to be efficacious in the management of certain adult stable diabetic patients, providing no evidence of toxicity is found.

The Acute Effects of DBI on Human Hepatic Intermediary Metabolism

By *Robert Tranquada, Charles Kleeman and Josiah Brown*. Department of Medicine, University of California Medical Center, Los Angeles, and V. A. Center, Los Angeles.

In an effort to determine the acute effects of DBI (phenethyldiguanide) on hepatic metabolism in vivo, 5 diabetic patients, 2 following pancreatectomy and 3 with chronic pancreatic insufficiency, were studied by means of right hepatic vein catheterization. Patients were fasting and without regular or long-acting insulin for 12 or 48 hours, respectively. After a control period, 150 mg. DBI was given by mouth, and femoral arterial and hepatic vein concentrations of oxygen, BUN, glucose, lactate, pyruvate and BSP were determined simultaneously every ½ hr. for 4 hrs. Estimated hepatic blood flow (EHBF) was determined by the BSP method.

Five subjects were followed for a 4 hr. period after DBI. Arterial glucose fell after 1½ hrs. Hepatic urea and glucose production and oxygen consumption showed no significant change in 4 hours. Arterial lactate and pyruvate levels showed no significant change, nor did hepatic lactate and pyruvate production increase. In fact, there appeared to be an increased splanchnic removal of pyruvate and lactate partly as a result of an average increase of 30% in the EHBF during the period studied.

The fall in arterial glucose in the absence of decreased hepatic glucose production suggests

increased peripheral utilization of glucose. Failure to find increased lactate production or decreased BUN production by the liver is in disagreement with previous findings as is the lack of change in O_2 consumption. These results suggest that, within these time and dose limits, the chief activity of DBI is the stimulation of increased peripheral glucose utilization with increased peripheral production of lactate and pyruvate which are metabolized in the splanchnic circulation.

Tissue Distribution of Administered DBI and Its Relationship to DBI Action

By *Arne N. Wick and Charles J. Stewart.* San Diego State College.

The biguanide commonly referred to as DBI has a marked hypoglycemic effect in vivo. The in vitro studies have shown that DBI will inhibit the oxidation of fuels such as acetate, lactate and glucose. Considering these facts by themselves, one would not expect DBI to have a hypoglycemic action. In order to investigate this paradox, C^{14}-labeled DBI [N^1-(2-phenyl-1-C^{14}-ethyl)-formamidinyliminourea hydrochloride] was administered at 100 mg./Kg. orally and intraperitoneally to 24-hour-fasted rats. The rats were sacrificed at the end of 1, 2, 5, 8, 12 and 24 hours. Samples of muscle, liver, kidney, heart, lung, spleen, adipose, gastrointestinal tract, blood, urine and expired CO_2 were isolated and examined for their C^{14} content.

Over 90% of the administered C^{14} was excreted in the urine within 24 hours after both oral and IP administration. No C^{14} was found in the expired air. In the shorter time periods the liver and gastrointestinal tract concentrated the C^{14}, almost to the exclusion of other tissues. The high concentration of C^{14} found in the gastrointestinal tract follows the pH partition hypothesis of gastric secretion of basic drugs (Parkhurst et al.). It is believed that the high concentration of C^{14} (presumably DBI) found in the liver is sufficient to inhibit the citric acid cycle function (Wick et al.) and thereby favor an increase in the conversion of glucose to lactic acid in this tissue. The lactic acid thus produced is then utilized by the peripheral tissue. By this mechanism, DBI may operate beneficially in a manner not found in nature.

The Effect of the Acute and Chronic Administration of Hydrocortisone on the Release, Inactivation and Action of Antidiuretic Hormone (ADH)

By *Charles R. Kleeman, Jerry Koplowitz, Morton H. Maxwell and J. Thomas Dowling.* Department of Medicine, V. A. Center, and University of California Medical Center, Los Angeles.

The impaired water diuresis of adrenal insufficiency may be completely corrected by hydrocortisone administration. Studies from this laboratory indicate that this correction is not due to an improved glomerular filtration rate or an altered tubular reabsorption of solute. They suggested a direct effect of hydrocortisone on the diluting segments of the nephron; however, an effect on the metabolism of ADH was not ruled out.

The effect of hydrocortisone on ADH metabolism was investigated in 9 normal subjects and 3 diabetes insipidus patients. Paired studies with and without acute or chronic (3–5 day) hydrocortisone administration were run on each subject.

Results: The acute or chronic administration of hydrocortisone (200 mg. daily) had no effect on the following responses involving ADH in normal subjects: (1) the antidiuresis following hypertonic saline administration or venous congestion of the lower extremities during water diuresis; (2) the maximal antidiuretic response to 18 hours of dehydration with or without Pitressin administration; (3) the length of time necessary to attain maximal diuresis following a sustained water load or to return to normal flow following water withdrawal.

Hydrocortisone (chronic administration) caused a significant augmentation of the maximal water diuresis of diabetes insipidus subjects (greater flow and free water clearance and lower minimal urinary osmolality).

Conclusions: Hydrocortisone had no effect on the release, inactivation or action of ADH in normals. Hydrocortisone can augment maximal water diuresis in the absence of ADH (diabetes insipidus). Hydrocortisone probably improves diuresis in adrenal insufficiency by a direct effect on the renal tubule.

Effects of Radiation Therapy on Adrenocortical Function

By *Robert S. Cox, Jr., Ward A. Soanes and John R. Maher.* Research and Development Service and Urology Service, Letterman Army Hospital, San Francisco, and Chemistry Branch, U. S.

Army Area Medical Laboratory, Fort Baker, California.

The study was undertaken to evaluate the effects of therapeutic doses of deep x-ray therapy to the region of the adrenals on adrenal function as measured by steroid excretion studies and the response of the adrenals to ACTH stimulation. Nine cases of testicular tumor treated with abdominal radiation, including the adrenal areas, have been studied by determining 17-ketosteroid and 17-hydroxysteroid excretion in the urine. Base line steroid levels and levels of response to 40 units of i.v. ACTH were obtained, and thereafter almost daily steroid excretion levels and periodic ACTH stimulation studies were carried out during and following irradiation. In most cases, follow-up levels were obtained 1 to 3 months following therapy. The patients received 2500 r to 3500 r tumor dose to both adrenals over a period of approximately 30 days.

The studies show no significant differences in basal steroid excretion values. During irradiation and following it there is a tendency for increased adrenal response to ACTH stimulation, but stimulation studies 30 or more days following therapy again returned to approximate base line levels.

This study shows that the administration of therapeutic doses of deep x-ray therapy of as much as 3500 r to the adrenal glands does not appreciably affect adrenocortical activity, but the adrenal response to stress, as demonstrated by ACTH stimulation, is transiently increased. These results are substantiated by almost daily studies on 9 patients.

Steroidogenesis by Adrenal Adenomata and Nontumorous Adrenal Tissue in Vitro

By *C. I. Slade, R. E. Bailey, A. H. Lieberman and J. A. Luetscher, Jr.* Department of Medicine, Stanford University School of Medicine, San Francisco.

Adrenal adenomata and nontumorous adrenal tissue removed surgically from 2 cases of mineralocorticoid syndrome, 2 cases of Cushing's syndrome and 1 case of idiopathic edema have been incubated in order to study the tissue's secretory potential and responsiveness in vitro. Tissue slices were incubated in Krebs-Ringer bicarbonate buffer equilibrated with 95% O_2 and 5% CO_2 and found to secrete corticosteroids for many hours. Of the several compounds extracted from the incubation media, 3 have been identified as aldosterone, cortisol and corticosterone. Corticosteroid secretion was considerably increased by the addition of human serum or corticotrophin to the incubation media.

In mineralocorticoid syndrome due to adrenal adenoma, cortisol and corticosterone were recovered in considerably greater amount than aldosterone from the adenoma incubates. In Cushing's syndrome due to adrenal hyperplasia, cortisol was the predominant secretory product. In idiopathic edema, cortisol was likewise the steroid recovered in greatest amount, together with smaller quantities of aldosterone.

More definitive studies are necessary to determine how closely the pattern of secretion in vitro resembles that of the same tissue in vivo. At present, incubation can indicate potential secretion and responsiveness of isolated adrenal tissue or tumors and possibly provide information not otherwise available regarding source and nature of hormonal derangement in disease states.

Evaluation of the Metabolic Effects of Dexamethasone

By *Stanley Newman, David Dorosin and Vincent DiRaimondo.* Department of Medicine, Metabolic Unit for Research in Arthritis and Allied Diseases, University of California School of Medicine, San Francisco.

16-Alpha-methyl, 9-alpha-fluoroprednisolone (Decadron), a new glucocorticoid, was found to be 25–40 times as potent as hydrocortisone in suppressing endogenous ACTH in 6 normal adult males.

The metabolic effects of 4 mg. dexamethasone daily were compared with 160 mg. hydrocortisone and 40 mg. triamcinolone in 3 mild diabetics and 1 patient without endocrine disease. All patients were hospitalized and kept on a constant dietary intake during the entire study. In the diabetics, there was a similar increase in fasting blood sugars, urinary glucose and urinary nitrogen during dexamethasone and hydrocortisone administration. There was little if any sodium retention with dexamethasone. In the nondiabetic patient the degree of increased nitrogen excretion, without apparent sodium retention or potassium loss, was similar to the closely related triamcinolone.

Dexamethasone has been employed recently as an anti-inflammatory agent. It is particularly useful, diagnostically and therapeutically, as an adrenal cortical suppressant in androgenic adre-

nal hyperplasia because of its increased potency associated with a lack of salt retention. However, the increased potency, as in the cases of the other corticosteroids, is accompanied by corresponding increases in carbohydrate and protein effects.

The Effects of Cold and Irradiation upon Corticoid and Ketosteroid Excretion in the Guinea Pig

By *Harry Sobel and Martin B. Sideman.* Institute for Medical Research, Cedars of Lebanon Hospital, and Department of Biochemistry and Nutrition, University of Southern California, School of Medicine, Los Angeles.

This laboratory is concerned with the metabolic consequences of age-associated disturbances in hormonal secretion. For example, in human physiology, with aging there is a decrease in androgen and urinary ketosteroid excretion, while that of the corticoids is relatively undisturbed. Chronic illness advances this pattern. Evidence of an antianabolic effect should become manifest when this disturbance becomes sufficiently advanced. An attempt is underway to develop an experimental tool for such studies. Although the guinea pig does not exhibit a loss of nitrogen following cortisone administration, it is a useful subject in which to study steroid excretion. Guinea pigs were placed in a cold room at 2–4 C. for 80 days. Ketosteroid excretion was relatively unaltered. Corticoid excretion, however, increased markedly and remained high for the duration of the time in the cold. The ratio of ketosteroid to corticoid fell from an average value of 4.7, prior to exposure to cold, to an average of 1.6 while in the cold room. Following removal to room temperature, corticoid excretion fell to normal and the ratio rose to 3.6. Guinea pigs were exposed to 300 r of x-rays. The survivors after 12 weeks exhibited a ketosteroid-corticoid ratio of 1.9, while unirradiated controls showed a ratio of 3.3. These findings suggest that the irradiated guinea pig may be a useful tool for the study of altered steroid production.

The Renal Excretion of Uric Acid in Patients with Gout and in Nongouty Subjects

By *C. A. Nugent and F. H. Tyler.* University of Utah College of Medicine, Salt Lake City.

There is general agreement that the renal excretion of uric acid in patients with gout is similar to that of normal subjects. However, the renal function studies which led to this conclusion were performed while the normal subjects and the gouty patients had markedly different plasma uric acid concentrations.

In this study, uric acid and inulin clearances were performed on 7 male patients with gout and 7 nongouty male subjects. In the gouty patients and the nongouty subjects, inulin clearances and uric acid clearances were in the same range, and, when studied under control conditions, about the same proportion of filtered uric acid was excreted. Increase in the plasma uric acid concentration of the nongouty subjects was induced by giving high purine diets or supplements of uric acid, ribose nucleic acid or desoxyribose nucleic acid for 3-day periods. At plasma uric acid concentrations comparable to those found in the gouty patients, 6 of the 7 nongouty subjects excreted a higher proportion of their filtered uric acid than did the patients with gout.

This difference between the gouty and nongouty subjects may be due to a difference in the renal handling of uric acid or a difference in the physical state of the plasma uric acid, or may be a transient phenomenon related to the short duration of the plasma uric acid elevation in the nongouty subjects.

Tissue Citrate as a Possible Guide to Changes in Intracellular pH

By *Belding H. Scribner, Michael A. Crawford and Malcolm D. Milne.* Department of Medicine, Postgraduate Medical School, London, England.

Studies to be published elsewhere suggested a relationship between tissue citrate and intracellular pH: In the rat, urinary citrate was shown to be relatively independent of the filtered load and to correlate with the level of citrate in kidney tissue. In addition, kidney tissue citrate was altered by factors which would be expected to alter intracellular pH; alkalosis increased and acidosis decreased kidney and urinary citrate levels.

In the present studies, changes in rat tissue citrate were measured in order to determine whether they would reflect changes in intracellular pH. Citrate was measured by the pentabromacetone method on a trichloroacetic acid filtrate of freshly ground muscle and kidney.

KCl (6 mm. Kg.) was injected intraperitoneally. Controls were injected with H_2O or isotonic NaCl. KCl caused a 40 to 50% rise in muscle

citrate at ½, 1½ and 2 hours, despite an extracellular acidosis as reflected by a 3–5 mEq./L. drop in serum bicarbonate. The urine remained acid. This rise in muscle citrate may reflect an intracellular alkalosis which might result from the displacement of H^+ by K^+.

Thirty minutes after intraperitoneal acetazoleamide (50 mg./Kg.) there was a slight decrease in muscle citrate, a 50 to 200% increase in kidney tissue citrate and a 2–4 fold rise in urinary citrate. Acetazoleamide, by blocking H^+ production, may have produced renal tubular alkalosis.

Three hours after acetazoleamide, urine and kidney tissue citrate fell below controls despite persistence of HCO_3^- in the urine, suggesting: (1) renal tubular cells became acid because of renal wastage of bicarbonate (serum bicarbonate fell 4–7 mEq./L.); (2) urine bicarbonate excretion following acetazoleamide continued despite renal tubular acidosis.

Gastrointestinal Absorption of Rubidium-86 in Hyperkalemia

By *Franz K. Bauer, Nancy Telfer and Marva A. Jenkins.* University of Southern California School of Medicine, and Los Angeles County Hospital, Los Angeles.

Potassium leaves cells during acidosis and starvation and raises the serum potassium level. Since it is frequently impossible to correct these 2 factors, it is common practice to withhold potassium from patients with oliguria or anuria and hyperkalemia.

This study was designed to find out whether potassium given orally is absorbed from the gastrointestinal tract in the presence of elevated serum potassium and whether it enters cells from the plasma.

Rubidium is handled by the body qualitatively similarly to potassium except for renal reabsorption. Rubidium-86 was chosen rather than potassium-42 because of its longer physical half-life. Tracer doses of 300 to 500 μc. were given orally in 25 to 100 ml. water. Serial whole blood and plasma samples were counted up to 3 days; fecal excretion of the tracer dose was measured and external counting over the entire body done after 24 and 48 hours.

Ten control patients without electrolyte imbalance and with normal urinary output; 7 patients with hypokalemia due to gastronasal suction, diarrhea and cirrhosis of the liver; and 8 patients with hyperkalemia due to renal impairment from sepsis, transfusion reactions, disseminated lupus, polycystic kidneys, ureteral obstruction and terminal chronic pyelonephritis and nephrosclerosis were studied.

Rubidium-86 was promptly absorbed from the gastrointestinal tract in all patients. No rubidium-86 could be recovered in the stools (less than .1 μc.). Whole blood radiorubidium concentrations in the hyperkalemic patient reached a peak in 24 hours, and were comparable to the control values of .4% to 1.0%/L. plasma. Plasma radiorubidium levels in the control patients reached their peak of .1% to .28%/L. plasma after 3 hours. In the hyperkalemic patients a peak of .25% to .55%/L. plasma was reached after 6–9 hours. After 24 hours the levels dropped but remained slightly higher than those of the control patients.

External counting over the body revealed high radiorubidium counts over the liver in all patients except in a terminal patient 3 hours before death.

It is concluded that rubidium-86 is absorbed from the gastrointestinal tract in patients with hyperkalemia and that it enters cells.

Comparative Analysis of I^{131} Albumin Metabolism and Distribution

By *Warren L. Beeken, Wade Volwiler, Patrick D. Goldsworthy, Patricia Ann Wood, Marion P. MacMartin and Chester Westort.* Department of Medicine, University of Washington, Seattle.

Various mathematical approaches to the analysis of data concerning protein metabolism and distribution have been suggested. To evaluate certain of these methods, 12 studies of I^{131} albumin metabolism and distribution were conducted in 8 normal, nonobese, young adult males.

A tracer dose of non-heat-treated, lightly labeled I^{131} albumin was administered intravenously, the plasma determined by isotope dilution, and the plasma and urine radioactivities followed for 28-day periods. Serial weight, hematocrit and serum albumin determinations indicated steady state of subjects.

Three methods of determining the extravascular/intravascular (EV/IV) albumin mass ratio were compared: (1) Extrapolation Method —the ratio was obtained from the EV/IV radioactivities at zero time found by extrapolation of the straight line portion of the IV radioactivity

curve. (2) Equilibrium Time Method—the EV/IV ratio was derived from the EV/IV radioactivities at equilibrium time (77 ± 25.3 hours after administration). Equilibrium time was estimated from values of EV radioactivity obtained by subtracting from the administered radioactivity the sum of the IV and cumulative urine radioactivities. (3) Transfer Rate Constant Method—the albumin mass ratio was determined from transfer rate constants by a method adapted to open mammillary systems by C. E. M. Matthews.

Method (1) assumes a system of 2-body compartments with albumin of uniform specific activity. Method (2) also assumes 2 compartments and requires calculation from data obtained before a constant rate of change of albumin distribution has been established. Method (3) involves none of these objections, and our data best fitted the 3-body compartment model of this analysis.

The mean metabolic half life of this I^{131} albumin was 15 ± 1.9 days. The mean EV/IV albumin mass ratio derived by method (1) was 1.8 ± 0.53 (mean of 3.1/1.8 Gm./Kg.). Method (2) gave a mean ratio of 1.4 ± 0.33 (mean of 2.2/1.8 Gm./Kg.). The mean ratio by method (3) was 1.3 ± 0.28 (mean of 2.2/1.8 Gm./Kg.). Despite the theoretical advantages of method (3), the derived ratios did not differ significantly from those of the other methods at 95% confidence levels.

Freely Extractable Plasma Lipid

By *George D. Michaels, Paul F. Flynn, Geoffrey Walker, Adolpho Barcellini and Laurance W. Kinsell.* Highland Alameda County Hospital, Institute for Metabolic Research, Oakland, California.

As part of work designed to develop a simple method for determination of chylomicronous fat in plasma, undenatured plasma was extracted with a variety of lipid solvents at pH 6. It was found that benzene extracted a fat fraction which appears to be of relatively specific composition in fasting blood, and which contains more than 80% of the unesterified fatty acids present in plasma. Protein denaturation is necessary if one is to recover 100% of the plasma unesterified fatty acids. On the basis of this finding, it would appear that by far the greater portion of the unesterified fatty acid is very loosely bound to protein.

In pre-heparin plasma, the unesterified fatty acids account for approximately 10% of the freely extractable lipid. The remainder of the freely extractable lipid is made up of cholesterol esters, glycerides, and free cholesterol, listed in order of magnitude. No phospholipid is present. In post-heparin plasma, the unesterified fatty acid of the freely extractable lipid is increased to a marked degree, and the other lipids decrease, percentage-wise and absolutely.

Plasmas from patients with grossly lipemic sera as part of one of the lipoidoses have much greater amounts of freely extractable lipids than normal plasma, and the composition is grossly different from normal.

It seems not improbable that freely extractable lipid is a "functional unit" of the circulating lipids. Under study at the present time is the composition of this entity with reference to mono-, di- and triglycerides, and with reference to fatty acid composition of each one of the lipids present, under conditions of fat loading (using saturated and unsaturated fat, respectively) in relation to heparin, and in patients with atherosclerosis and specific lipoidoses.

Lipide Synthesis in Normal and Obese Mice

By *D. D. Feller, E. Feist, R. L. Huff and N. R. Eaton.* V. A. Hospital, and University of Washington School of Medicine, Seattle.

The aim of this study is to determine if propionic acid is *selectively* incorporated into long-chain lipides by the fat depots of the intact mouse. Forty mice, fed ad libitum to time of experiment, were injected with sodium propionate-1-C-14 and sacrificed in groups at various intervals to 24 hours. Sixteen of these animals were made obese to varying degrees by previous treatment with goldthioglucose. The mice were kept in all-glass cages equipped to collect carbon dioxide, urine and feces. At sacrifice, total glycogen, nonsaponifiable lipides and fatty acids were recovered by standard procedures and C-14 content determined. The values expressed as per cent C-14 recovered in fatty acids obtained for each time interval were averaged and the mean value plotted. The fatty acid curve for obese mice reached a maximum value 4 times greater than the curve for normal mice. The peak of the obese curve occurred at 5 hours after injection while the peak for the normal curve was reached at 17 hours. By 24 hours, both curves fell to the same value. Nonmetabolized propionate recov-

ered in the urine and feces varied from 0.5% at the early intervals to 10% at the later ones. Glycogen and nonsaponifiable lipides accounted for negligible amounts of C-14, and the balance of C-14 was found in the expired CO_2. The results show that injected propionate is incorporated into lipides to a greater extent by fat animals. Furthermore, they indicate the rapid turnover that body fat may have.

Evidence for Control of Unesterified Fatty Acid Metabolism by the Sympathetic Nervous System

By *Richard J. Havel and Alan Goldfien.* Cardiovascular Research Institute, Metabolic Unit, and Department of Medicine, University of California School of Medicine, San Francisco.

Recent evidence indicates that epinephrine increases circulating levels of unesterified fatty acids (UFA). We have investigated effects of epinephrine, norepinephrine and sympatholytic agents and procedures to elucidate the role of the sympatho-adrenal system in fatty acid metabolism.

Studies were performed on fasting men, intact dogs and adrenalectomized dogs on replacement therapy. Drugs were administered intravenously. Plasma UFA concentration was estimated by the method of Davis.

In man infusion of 100 μg. epinephrine or norepinephrine markedly increased UFA concentration. Infusion of 0.5 μg./min. epinephrine increased UFA 0.60 μEq./ml. in 30 minutes without affecting plasma sugar concentration. In dogs, 0.05 μg./Kg. epinephrine or 0.25 μg./Kg. norepinephrine increased UFA concentration as early as 5 minutes after administration. Dogs pretreated with dibenamine (25 mg./Kg.) failed to show increased UFA concentrations following epinephrine or norepinephrine (10 μg./Kg.), although after epinephrine mean plasma sugar rose 0.77 mg./ml. In 5 studies, administration of hexamethonium (5 mg./Kg.) produced a fall of 0.40–0.75 μEq./ml. in UFA. Three-fourths of the fall occurred within 10 minutes. In 2 studies, subsequent insulin administration (0.3 U/Kg.) had no further effect. Similar changes followed epidural procaine or high cord section. Measurements of UFA, radioactivity and specific activity during concurrent administration of tracer albumin-bound palmitic acid-1-C^{14} and hexamethonium indicate that observed effects of hexamethonium result from a striking decrease in UFA inflow to plasma.

These studies support participation of the sympatho-adrenal system in control of fatty acid metabolism and suggest a relationship between the nervous system and lipid metabolism of far-reaching consequences.

Fatty Oxidation in Man as Affected by Nutritional States

By *Josiah Brown and Leslie R. Bennett.* Departments of Medicine and Radiology, University of California Medical Center, Los Angeles.

Oxidation of nonesterified fatty acids (NEFA) is an important and perhaps the major energy source in the fasting state. This study was designed to elucidate the effects of glucose administration and fasting ketosis on the rate of fatty acid oxidation in normal man.

Following intravenous administration of a tracer of C^{14}-labeled albumin-bound palmitic acid, the C^{14} content of the expired air was measured continuously for 1 hour. Total C^{14} output was measured by placing an air-tight hood over the head of the subject; a stream of compressed air carried the expired CO_2 out into an ionization chamber connected with a continuous recorder. Frequent blood samples were obtained through an indwelling needle in the antecubital vein for measurement of NEFA and glucose, the blood NEFA concentration serving as an index to the pool size.

Following control measurements, normal subjects were given glucose intravenously for 1–2 hours or fasted for 46 hours to produce ketosis, and the studies were repeated.

Glucose administration lowered blood NEFA levels to 1/5 of the control and decreased C^{14} output to ½. It is concluded that glucose administration decreased oxidation of fatty acids to a low level, confirming similar findings in the rat.

Prolonged fasting with ketosis increased blood NEFA levels 50%, with no change in C^{14} expired. Since outflow of the same quantity of tracer occurred from a larger pool, it is concluded that during fasting ketosis, fatty acid oxidation is increased.

GASTROINTESTINAL SYSTEM

Intraluminal Pressures in Patients with Gastroesophageal Reflux

By *Agostinho Bettarello, Stewart G. Tuttle and Morton I. Grossman.* Gastroenterology Section, V. A. Center, and Department of Medicine, University of California Medical Center, Los Angeles.

Simultaneous measurement of respiration, pressure and pH at various levels within the fundus and esophagus permits detection of gastroesophageal acid regurgitation, the height and length of the area of increased pressure at the hiatus and the magnitude of the decrease between mean expiratory fundic and mean expiratory intrathoracic pressure. Of 85 individuals studied, 45 manifested acid regurgitation. In this latter group, 42 showed an area of increased pressure at the hiatus averaging 6.62 cm. (H_2O) in height. In 38 of these, the average length of this zone was 2.13 cm. In the remaining 4, an increase in pressure was sustained throughout the esophagus, a phenomenon which was observed only in patients with reflux.

Among the 40 without regurgitation, 39 showed an increase in pressure at the hiatus averaging 6.76 cm. in height and 2.08 cm. in length. The magnitude of decrease between mean expiratory fundic and mean expiratory intrathoracic pressure was 5.66 and 5.46 cm., respectively, in the 2 groups. None of these differences was statistically significant.

Eight subjects who failed to manifest acid reflux were given 1.2 mg. atropine intravenously. Four of these individuals then showed marked regurgitation.

Thus, the occurrence of gastroesophageal reflux could not be correlated with absence or diminution of the zone of increased pressure at the hiatus, so a simple static failure of a "sphincter" cannot be held responsible for incompetence. However, the fact that atropine may induce reflux in normal subjects suggests that activity of smooth muscle is involved in prevention of gastroesophageal reflux.

Trypsinogen, Trypsin and Trypsin Inhibitor in Human Pancreatic Juice

By *Bernard J. Haverback, Hallie Bundy and Barbara Dyce.* University of Southern California School of Medicine, and Los Angeles County Hospital, Los Angeles.

In acute pancreatitis it is likely that trypsinogen is activated to trypsin within the pancreas by a mechanism not yet elucidated. Pancreatic juice contains at least one substance that inhibits the proteolytic action of trypsin. The purpose of the present study was to evaluate in human pancreatic juice: (1) trypsin inhibitor levels and (2) mechanisms which are responsible for the activation of trypsinogen to trypsin. Pancreatic juice was obtained by catheter drainage of the duct of Wirsung in 3 human subjects and from 2 others with pancreatic fistulae.

The method for determining tryptic activity was a modification of that developed by Karmen, utilizing the synthetic substrate benzoylarginine-paranitroanalide in a system at pH 7.6. The following results were obtained: Pancreatic juice had no spontaneous tryptic activity but contained trypsin inhibitor activity. Human enterokinase activated trypsinogen in juice, the over-all rate being proportionate to the amount of enterokinase added. When trypsin was added to pancreatic juice, in excess of the amount required to saturate the inhibitor, no autocatalysis occurred. Pancreatic juice was made 0.1 M with respect to calcium ions, and again no activation of trypsinogen to trypsin was observed. However, when both calcium ion and an excess of trypsin were added, conversion of trypsinogen to active trypsin occurred. Contrary to expectation, when pancreatic juice was incubated with calcium ion conversion of trypsinogen to trypsin by enterokinase was inhibited.

It was concluded that: (1) human pancreatic juice contains active trypsin inhibitor but no spontaneous tryptic activity; (2) the differences in the action of calcium ion on the activation of trypsinogen in human pancreatic juice by enterokinase and by trypsin, indicate the importance of ion binding at or near the site of activation.

Amino Acid Patterns in Arterial and Hepatic Venous Blood

By *Sherman M. Mellinkoff, Telfer B. Reynolds, Marjorie Frankland and Margaret Greipel.* Department of Medicine, University of California (Los Angeles) Medical School, and

Department of Medicine, University of Southern California School of Medicine.

While the total blood amino acid concentration rises enormously following hepatectomy, profound alterations in hepatic function (coma, for example) may appear without changing total blood amino acid concentration. Under these circumstances, however, the blood amino acid pattern is often abnormal. It is therefore important to know what happens to the concentration of individual amino acids as they traverse the liver.

In 14 patients with Laennec's cirrhosis ranging in severity from very mild to severe, and in 2 patients without liver disease, a total of 30 simultaneous paired blood specimens were obtained from one femoral artery and from the hepatic vein. Each specimen was analyzed by means of 2-dimensional paper chromatography, with phenol and ter-butyl alcohol as solvents and ninhydrin the color developer.

Results were variable from patient to patient and in so small a series could not be correlated with functional impairment. The only consistent changes (found in almost all subjects) were a marked rise of glutamic acid and fall of alpha-alanine in the venous as compared with arterial blood. Different changes were found and definitely confirmed in some patients, however. The one patient with a relatively enormous venous-arterial ratio of glutamic acid had Korsakoff's psychosis.

It is concluded that the liver can extract from or add to the blood passing through it many amino acids individually. It is probable that these alterations in amino acid pattern change in response to metabolic circumstances.

Blood Ammonia Dynamics in Hepatic Encephalopathy and the Influence of Miscellaneous Experimental Therapy

By *Daniel H. Labby and Jack H. Hutchinson.*

Continuing studies of the behavior of blood ammonia concentration have been aimed at clarifying their dynamics during the stages of hepatic encephalopathy. Data have now been derived from observations on 120 patients. In addition to establishing the greater clinical dependability of the arterial ammonia concentration over the venous, systematic studies of A-V differences have disclosed the earliest changes in the pre-coma state to be that of a rapidly rising arterial concentration and a lagging venous rise. In complete coma, the new steady state is not one of saturation with small A-V differences as frequently reported, but rather one of an equilibrium most commonly marked by large A-V differences, though at excessive blood ammonia concentrations. These data are at variance with information currently available in the literature. With effective treatment, the first (and most rapid) fall occurs in the venous ammonia level, shortly followed by a fall in the arterial, frequently further increasing the A-V difference. Return to normal dynamics occurs later with full recovery; with relapse, the cycle is repeated—stabilization at levels in excess of normal is fatal. The influence of miscellaneous ammonia-binding treatment agents reveals no major advantages among them, with the exception of a few at critical intervals.

Evidence for the Metabolism of Bromsulfalein (BSP)

By *John V. Carbone, G. M. Grodsky and R. Fanska.* Department of Medicine, University of California School of Medicine, San Francisco.

Despite the widespread clinical use of BSP clearance as a liver function test, there is no proof of what liver function(s) is actually involved in the clearance of the dye. Supposedly, a BSP complex with protein formed in the blood is broken in the liver, and free BSP is excreted into the bile. We have attempted to determine whether a biochemical function is involved in BSP excretion from the liver. Protein-free acetone extracts containing BSP from human bile or urine were subjected to chromatography in t-butanol-water. Two, possibly 3, slow-moving pigments were distinguishable from free BSP. These pigments, representing altered BSP products, constitute 65–75% of the total BSP appearing in bile or urine. In perfusion studies with isolated liver, these BSP metabolites accounted for 90% of the pigment appearing in the bile. BSP-A, the most abundant metabolite, has been further purified by paper electrophoresis and by chromatography in n-butanol-acetic acid. The absorption spectra and dissociation constant of this product are indistinguishable from free BSP. BSP-A has proved stable to hydrolysis in dilute HCl, dilute NaOH and incubation with β-glucuronidase and gives a negative Dische reaction. The metabolite was found to be ninhydrin positive and unstable after treatment with 6N HCl at 120°. Amino acid complexes with BSP have been synthesized, and their characteristics will be compared to the natural compound. It is concluded that BSP is metabolized in the liver and excreted as a conjugate which is not a glucuronide or an ethereal sulfate

but may be a complex with a ninhydrin-positive moiety.

The Metabolism of Sulfobromophthalein (BSP) in the Human

By *Lee S. Monroe and Alice Kittinger.* Scripps Clinic and Research Foundation, La Jolla, California.

Relatively little is known of the mechanism of transfer of sulfobromophthalein (BSP) from the blood into the bile. Possibly this is because workers have assumed that since BSP excreted in the bile retains its quality as an indicator, the molecular structure has not been modified. In animal experiments, however, Krebs and Brauer have demonstrated by chromatographic technics and studies with S^{35}-labeled BSP that the dye undergoes changes in the process of excretion. Using a modification of the method of Krebs, chromatographic studies employing cationotropic alumina were performed on BSP obtained from the bile and urine of postoperative human subjects. Bile-type BSP was observed to chromatograph in 4 fractions, 3 of which are separate from pure BSP. Urine-type BSP usually chromatographs in 3 fractions, 2 of which can be separated from pure BSP. Similar chromatographic studies of urine-type BSP have been made in a variety of patients with liver disease and jaundice. These chromatograms show variation and may be of clinical value when the transfer mechanism of BSP is understood.

A BSP derivative found in bile and urine and separate from pure BSP can be demonstrated by ascending chromatography with acetone: propionic acid: water. This changed BSP is ninhydrin-positive. The possibility that this represents an amino acid conjugate is under investigation.

No evidence has been found in human studies that the formation of a glucuronide explains the bile-type BSP observed.

Effects of Bromsulfalein on the Cardioportal Circulation Time

By *Ismael Mena, Leslie R. Bennett and R. Wilbur Melbye.* Department of Radiology, University of California, School of Medicine, Los Angeles.

The possibility of an intravenous injection of BSP producing acute hemodynamic changes in the liver has been studied by measuring the cardioportal circulation time.

The cardioportal circulation time is determined by scintillation detectors externally placed over the heart and over the liver. The heart detector feeds its information to an EPUT meter (events per unit time); the liver detector is connected to a rate meter–recorder unit. In a fasting individual 0.25 μc./Kg. of radioiodinated serum albumin (RISA) is injected rapidly into an antecubital vein. Recording of radioactivity over the heart and the liver is begun at the moment of RISA injection and is concluded when a well-defined plateau of radioactivity is reached over the liver. Both the cardiac and the liver data are plotted on the same graph. The cardiac curve has proven to be a meaningful reference point from which to determine forward circulation times. For this reason the time between the heart peak and the liver peak is measured and defined as the cardioportal circulation time. In normal individuals this time is 25 $\pm$ 3.04 seconds. In cirrhotic individuals this circulation time is 46 $\pm$ 5.6 seconds.

Twenty fasting, normal individuals were tested before, 2 minutes after, and 10 minutes after an intravenous injection of 2.5 mg./Kg. of BSP. In 60% of the normals the circulation time 2 minutes after injection was delayed to mean 40.8 $\pm$ 5 seconds, an abnormal range characteristic of that seen in cirrhotic patients. But after 10 minutes the circulation time returned to normal, mean 29.8 $\pm$ 3.9. The other 40% were unaffected by BSP.

In 10 cirrhotic patients the mean cardioportal circulation time was 39.4 $\pm$ 3 seconds. Two minutes after BSP injection, the circulation time in 8 patients was delayed to 51.14 $\pm$ 7 seconds. Ten minutes after injection it was still delayed 47.8 $\pm$ 7 seconds.

It is concluded that after an injection of BSP, 60% of the normal individuals studied showed evidences of acute liver hemodynamic changes characteristic of cirrhotics. In cirrhotic individuals the cardioportal circulation time was markedly delayed by the injection of BSP.

Primary Biliary Cirrhosis Treated with Ethyl Linoleate

By *Geoffrey Walker, Paul F. Flynn, George Fukayama and Laurance W. Kinsell.* Institute for Metabolic Research, Highland Alameda County Hospital, Oakland, California.

A woman with primary biliary cirrhosis, who had been jaundiced for 2 years and had had widespread xanthomata for 1 year, was treated for 13 weeks with a formula diet providing 80 Gm. ethyl linoleate daily and has subsequently

followed a diet containing 160 Gm. safflower oil (approximately 110 Gm. linoleic acid) as the sole source of fat.

Subjective improvement and reduction in the size of the xanthomata was evident within 6 weeks, and only a few lesions remained after 8 months.

The following plasma changes were observed (data given in the order of *before treatment, after 3 months, after 6 months, after 8 months*): Total lipids mg./100 ml.—3900, 1388, 1075, 1059; cholesterol mg./100 ml. (total)—1490, 398, 315, 284; (free)—1340, 286, 129, 104; (ester)—150, 112, 186, 180; (E/F ratio)—0.11, 0.39, 1.44, 1.73; phospholipids (as lecithin) mg./100 ml.—2520, 625, 435, 397; trihydroxy cholate μg./ml.—34, 44, 13, 27; dihydroxy cholate μg./ml.—36, 55, 20, 22; bilirubin mg./100 ml.—5.8, 5.6, 3.6, 2.6; alkaline phosphatase K.A. u./100 ml.—64, 48, —, —; cholesterol ester dienoic acid % of C.E.F.A.—22, 62, —, —.

Is the Intestinal Pathology of Celiac Disease Reversible? A Preliminary Report

By *Cyrus E. Rubin, Lloyd L. Brandborg and Patricia Phelps.* Division of Gastroenterology, Department of Medicine, University of Washington, Seattle.

To investigate the course of the intestinal lesion in celiac disease, 130 small bowel biopsies were performed in 32 cases and 32 controls.

A 4 mm. flexible tube was designed, one which enabled safe biopsy in infants and adults. After precise orientation and serial sectioning, the surface area was estimated by a modified Chalkley technic. Three separate observers evaluated the histology blindly.

Juvenile, "cured" and adult celiacs (1 to 55 years) showed the classical lesion in 26 and no lesion in 6. The histologic severity had no apparent relationship to the activity of the disease or its duration. No mucosal improvement was seen in 1 new celiac who was biopsied before and after 6 months of a gluten-free diet. Severe mucosal abnormalities were observed in patients who had been treated with the gluten-free diet, cortisone or nothing at all. All degrees of severity were demonstrable in all age groups, although there was a tendency to a milder lesion in the young.

These data suggest that the intestinal pathology in celiac disease usually persists throughout life, even during remissions. It is impossible to state whether the 6 patients with normal biopsies represent a reversion to normalcy or a past incorrect diagnosis; they were all clinically well when biopsied. The final answer to the question of reversibility will only be given by long-term serial biopsy study.

The Use of an Intramucosal Test to Demonstrate Food Hypersensitivity in Ulcerative Colitis

By *J. Alfred Rider, Hugo C. Moeller, Richard G. Devereaux and Robert R. Wright.* Departments of Medicine and Pathology, University of California Medical Center, San Francisco.

The etiology of ulcerative colitis is unknown, although many factors have been implicated and numerous treatments have been used with varying success, ranging from psychotherapy, antibiotics and chemotherapeutic agents to elimination diets and steroids.

The development of hypersensitivity of the colonic mucosa to something in the diet could partially explain at least some of the cases of ulcerative colitis. This concept is supported by the demonstration of changes in the basement membrane in the colon and the fact that cortisone and its derivatives usually produce a remission. To the present, there has been no objective way to determine whether patients with ulcerative colitis are hypersensitive to certain foods.

A method of intramucosal testing through the proctoscope by means of a special 5-inch 24-gauge needle with an attached adjustable guard was devised. Test solutions of 1:10 dilutions of wheat, eggs or milk were injected in 0.2 ml. amounts intramucosally, and the injected site was observed and compared with a control injection site for 20 minutes. Twenty-four hours later, both sites, which had been marked with India ink, were biopsied and examined microscopically.

Gross positive findings consisted of immediate erythema at the site of injection with engorgement of surrounding capillaries, followed within 2–5 minutes by the development of an edematous wheal 1–2 cm. in diameter.

Histologically, there was an acute vasculitis with swelling of endothelial cells, fibrinoid necrosis of the vessel walls and a perivascular inflammatory cell infiltration containing eosinophils. This response highly suggested an allergic reaction.

Although this test obviously cannot be used in acute ulcerative colitis, it can be used during relative remissions. Further experience with this test may enable us better to understand the etiology of ulcerative colitis.

GENITAL TRACT

Uterine and Fetal Blood Flow and Oxygen Consumption in Early Human Pregnancy

By *N. S. Assali, L. Rauramo, T. Peltonen and C. Gemzell.* University of California at Los Angeles, University of Turku, Finland, and Karolinska Sjukhuset, Stockholm, Sweden.

Study of the rate of blood flow and oxygen consumption of the pregnant uterus and fetus was performed on 12 normally pregnant women undergoing therapeutic abortion by hysterotomy. The length of gestation varied from 10 to 28 weeks. Uterine blood flow was measured with the N_2O technic and with a miniature electromagnetic flowmeter attached to one uterine artery. Fetal blood flow was measured by exteriorizing a small segment of the umbilical vein and inserting it into a miniature electromagnetic flowmeter before separation of the placenta and delivery of the fetus. Oxygen consumption of the total pregnant uterus and of the fetus was computed from the A-V oxygen difference and blood flow.

Uterine blood flow increased from 120 ml./min. at the 10th week of gestation to 240 ml./min. at the 28th week. However, when calculated on the basis of per 100 Gm./min., the flow did not change significantly. Oxygen consumption of the pregnant uterus changed in a similar fashion. Uterine arterial oxygen saturation varied from 82 to 96% and venous saturation from 75 to 87%. Fetal blood flow increased from 11 ml./min. at the 10th week to 80 ml. at the 28th week, but remained constant when calculated on the basis of per Kg. of body weight. Fetal oxygen consumption changed in a similar fashion. Fetal arterial oxygen saturation varied from 52 to 65% and venous from 33 to 45%.

It is concluded: (1) That the uterine circulation adjusts itself closely to the growing fetus and placenta and to the progressively enlarging pregnant uterus; (2) that despite this adjustment and despite a normal uterine oxygen saturation, the fetus lives in a hypoxic state *in utero*.

IMMUNOLOGY

Studies on a Wasting Disease Induced in Hybrid Mice Injected with Parental Strain Lymphoid Cells

By *H. S. Kaplan, B. H. Rosston and R. Skahen.* Department of Radiology, Stanford University School of Medicine, San Francisco.

Injection of thymus, spleen or lymph node cells from C57BL mice into sublethally irradiated F_1 (C57BL x BALB/c) mice elicits within 2 to 5 weeks a frequently fatal illness characterized by a sharp fall in body weight and diarrhea. Autopsy reveals profound depletion of body fat, a necrotizing, granulomatous reaction replacing the lymph nodes and splenic white pulp, extreme thymic atrophy, adrenal hypertrophy and variable lesions in the bone marrow, kidneys, liver and intestine. Preterminal blood cultures have been sterile. Laboratory findings include a severe anemia, leukopenia in excess of that due to irradiation and a decreased serum protein concentration, with virtual disappearance of the gamma globulin peak on paper electrophoresis. The condition seems self-limited: some animals gain weight again and appear healthy; when sacrificed later, these reveal hyaline scars and renewed lymphocytopoiesis in the lymphoid tissues. Prior adrenalectomy of the hybrid host leads to a milder, seldom fatal form of the disease, presumably by virtue of a lymphocytotrophic influence.

The underlying pathogenic mechanism of the disease is an immunologic reaction of donor versus host lymphoid cells. Cells from 1- to 4-day-old donors are ineffective. Spleen and lymph node are more active sources than thymus and will even elicit the disease in (very young) non-irradiated hybrids. Cells from BALB/c donors are less active than those from the C57BL parental strain.

Immunologic Studies in the Connective Tissue Diseases

By *W. J. Fessel, Wallace V. Epstein and Ephraim P. Engleman.* Rheumatic Disease Group, Department of Medicine, University of California School of Medicine, San Francisco.

The sera of patients with systemic lupus erythematosus, and occasionally other diseases of

connective tissue, often contain lupus factor, which is responsible for L.E. cell formation. Using latex particles sensitized with calf nucleoprotein and an extract of human aortic intima, we have tested sera for the presence of lupus factor.

With the latex nucleoprotein system, only 3 of 150 sera from normal persons caused agglutination. Twenty-five of 37 sera from patients with systemic lupus were positive. Of the 25 positive sera, 22 caused L.E. cell formation. Thirteen of the 25 also gave positive F II hemagglutination reactions for rheumatoid factor. Thirty-three of 150 sera from rheumatoid arthritics showed positive nucleoprotein latex reactions; 2 of the 33 caused L.E. cell formation.

When the latex was sensitized with a saline extract of aortic intima, 7 sera from patients with systemic lupus were positive. Four of 8 sera from patients with rheumatoid arthritis and clinical arteritis were positive. One of 8 sera from rheumatoid patients without obvious arteritis, but with high F II titers, was positive. Four of 5 sera from patients with polyarteritis nodosa were positive.

Multiple immunologic abnormalities are probably involved in the connective tissue diseases. One of these gives a positive reaction to the nucleoprotein latex test. This test, although less specific than the L.E. cell test, is much more easily performed and allows some quantitation of the amount of lupus factor present.

INFECTIOUS DISEASE

Bacterial Polysaccharide Binding with Plasma Proteins

By *Russell S. Jones.* Department of Pathology, University of Utah, College of Medicine.

The slow clearance of *K. pneumoniae* polysaccharide from the blood stream, and the inactivation of the ACTH-releasing property of the polysaccharide by incubation with plasma, may be related to the binding of polysaccharide with plasma proteins. By means of paper electrophoresis, considerable binding of C^{14}-labeled *K. pneumoniae* polysaccharide complex to gamma globulins in man, guinea pig, rabbit, rat and mouse has been demonstrated, and marked differences in binding to albumin of these species has been found. While the plasma albumin of the rat and mouse bound bacterial polysaccharide in vitro, albumin of the guinea pig did not. In a comparison of in vitro and in vivo polysaccharide-protein binding, an increase in the albumin binding was found after the intravenous injection of the C^{14}-labeled polysaccharide in the guinea pig. Studies have not disclosed whether the albumin binding was due to changes in the protein, the polysaccharide or in moieties associated with either. In the guinea pig, the factor(s) responsible for the in vivo albumin-binding apparently is not associated with leukocytes or tissues, heparin-lipemia clearance or proteolytic enzymes. Patients with different diseases showed considerable variation in the relative binding of the labeled bacterial polysaccharide to plasma proteins. Albumin binding of the bacterial polysaccharide was very slight in terminal disease states and obstructive jaundice and was increased in many chronic illnesses. Such findings suggest that albumin-binding of bacterial polysaccharide may have application as an index of host-response.

Postsplenectomy Severe Infections in Infants and Children: Relation to Disease, Postoperative Interval and Age

By *Tom W. Robinson and Phillip Sturgeon.* Division of Hematology, Los Angeles Childrens Hospital, and Department of Pediatrics, University of Southern California School of Medicine.

From 1935 through 1956, 148 splenectomies were performed. Contact with the individual or his physician enabled us to follow 112. The median interval between splenectomy and follow-up was 6 years, the minimum 1 and the maximum 22. Fatal or life-threatening infections occurred 12 times.

In 63 patients, their disease probably did not compromise resistance to infection. This included 37 with hereditary spherocytic anemia, 14 with idiopathic thrombocytopenic purpura and 12 with ruptured spleens; 19 were under 2 years at splenectomy. The single severe infection in these 63 was influenza meningitis, 2 months following splenectomy in a 4.5-month-old infant.

In the 69 remaining, the diagnoses, number of cases and number of infections (in parenthesis) follow: Cooley's anemia, 6(1); portal hypertension, 7(1); malignant tumors, 6; ac-

quired hemolytic anemia, 6(1); Gaucher's disease, 6(1); R.E. malignancies, 14(3); hypoplastic anemia, 7(2); miscellaneous, 18(2). All infections occurred within 2 years of splenectomy; 7 were fatal, 6 resulted from pneumococcus, 5 patients were less than 2 years old at splenectomy.

This analysis indicates in diseases without a basic predisposition to infection that the post-splenectomy risk of severe infection under 2 years of age may approximate 5%; over 2, it is minimal. In diseases predisposing to infection the risk approximates 10–15%.

Treatment of Urinary Tract Infections with Cycloserine

By *Paul D. Hoeprich and John R. Ward.* Department of Internal Medicine, University of Utah College of Medicine, and Salt Lake County General Hospital, Salt Lake City.

Cycloserine: (1) has broad antibacterial potential; (2) following oral administration, is quantitatively absorbed, rapidly distributed in all body fluids and excreted in the urine. Fourteen adult, hospitalized patients with urinary tract infections were given 0.250 Gm. cycloserine every 6 hours. In addition to clinical evaluation, before, during and after treatment: *A.* The urine was examined by: (1) Culture-quantitative; qualitative, with tube dilution antibacterial agent susceptibility testing of isolates. (2) Sediment inspection-quantitation of casts and cells; qualitative characterization of casts and cells by means of stains. (3) Determination of cycloserine urine excretory pattern. *B.* The blood was analyzed for concentration of: (1) Blood urea nitrogen; (2) creatinine; (3) cycloserine.

Four patients with indwelling urinary catheters showed temporary decrease in magnitude of bacteriuria and qualitative change in bacterial flora.

In 3 patients, confusion, disorientation and extreme drowsiness necessitated discontinuation of therapy after 48 to 72 hours; all 3 had azotemia before treatment and attained unusually high blood levels of cycloserine. Forty-eight to 72 hours after cycloserine was discontinued, there was return to pretreatment mental status.

The remaining 7 patients were given cycloserine for 7 or 14 days. In one patient, cycloserine was ineffective (pretreatment bacteriuria 280,000/ml.; after 14 days therapy, 28,800/ml.). Cycloserine was effective in 6 patients. White cells in the sediment decreased in number and no longer displayed the appearance of cells associated with active infection. Cultures either became sterile or yielded fewer than 2,000 bacteria/ml. Follow-up studies range from 1 to 6 weeks after cycloserine therapy was discontinued.

The Reiter Protein Complement Fixation (RPCF) Test in the Diagnosis of Syphilis

By *James N. Miller, Ruth A. Boak and Charles M. Carpenter.* Department of Infectious Diseases, School of Medicine, University of California at Los Angeles.

Although the Treponema pallidum immobilization (TPI) test has become established as the most specific procedure yet developed for the diagnosis of syphilis, the technical complexities involved in its performance preclude routine use in clinical and public health laboratories. The intensive efforts of investigators to find simpler procedures with a sensitivity and specificity comparable to the TPI test have led to the development of the Reiter protein complement fixation (RPCF) test. The procedure, originated by D'Alessandro and Dardanoni in 1953, utilizes as antigen, in a 1/5 volume Kolmer technic, a soluble, specific protein extracted from the avirulent Reiter strain of *T. pallidum*. Preliminary investigations in our laboratory on well-documented samples have indicated a sensitivity and specificity comparable to that of the TPI test. As a result, an extensive cooperative survey study was begun of: (1) accuracy of performance of the RPCF test in clinical and public health laboratories; (2) comparison of results of RPCF and TPI on sera from patients with no history of syphilis and reactive serologic tests for syphilis (STS); (3) comparison of results of nonspecific tests (VDRL and Cardiolipin Kolmer) with the results of the specific tests (TPI and RPCF).

Serum samples routinely submitted to the UCLA laboratory for TPI tests were coded, divided into 4 aliquots and distributed to the participating laboratories. The Los Angeles City Health Department performed the VDRL and Cardiolipin Kolmer tests, the UCLA laboratory performed the TPI test, and all laboratories carried out the RPCF test utilizing antigen supplied by the UCLA laboratory. To date, results are available on 446 of 1000 samples to be tested. The favorable results obtained have led to a proposed plan for the serologic differentiation of B.F.P. reactions from those due to infection with *T. pallidum*.

Studies of Genetic Factors Influencing Isoniazid Blood Levels in Humans

By *H. Wm. Harris, Ralph A. Knight and Merle J. Selin.* Microbiology Research Laboratory, V. A. Hospital, and Department of Medicine, University of Utah College of Medicine, Salt Lake City.

INH (Isoniazid) levels were determined by bioassay on serum obtained 2, 4 and 6 hours after a 4 mg./Kg. oral dose. In 100 American-born individuals of European ancestry ("Caucasians"), the 6-hour serum levels ranged between 0.0 and 3.2 μg./ml. The levels were 0.2 μg./ml. or less in 40 (henceforth called "rapid inactivators"); levels of 0.8 μg./ml. or greater were found in 52 ("slow inactivators"); levels of 0.4 μg./ml. were found in 8 subjects. A curve plotting the distribution of serum values among the 100 subjects demonstrates marked bimodality rather than the bell-shaped "normal" curve of biologic variation. The mean serum levels of the "rapid" and "slow" inactivators differ significantly at 2, 4 and 6 hours.

Serum INH levels in 25 Americans of Japanese ancestry were compared to those of 25 Americans of European ancestry. Of the Japanese subjects, 23 (92%) were "rapid inactivators" in contrast to 12 (48%) "rapid inactivators" in the control group. A statistically significant difference was found between the numbers of "rapid" and "slow" inactivators, and between the mean INH levels in the 2 races.

Six-hour INH serum levels were determined on 36 parents and adult offspring of 5 "Caucasian" families. The data are insufficient for final conclusions but suggest that a low INH serum level ("rapid inactivation") is inherited as a simple dominant trait, while "slow inactivation" is inherited as a recessive trait.

KIDNEY

Creatine Synthesis in Nephrectomized Rats

By *Ralph Goldman and John X. Moss.* Medical Service, V. A. Hospital, Sepulveda, California, and University of California Medical Center, Los Angeles.

On the basis of in vitro studies, it has been believed that arginine-glycine transamidination occurs in the kidney, and that the glycocyamine formed is then methylated in the liver to form creatine. In order to study the possible effect of renal disease on creatine formation it was necessary to confirm in vivo the importance of the kidney in the transamidination reaction. Nephrectomized rats were maintained for up to 17 days by the peritoneal dialysis method of Kolff and Page. Muscle and carcass were assayed for creatine content at various intervals after nephrectomy. The creatine concentration increased slightly for the first few days, then decreased slightly after 2 weeks. The serum creatinine also increased for 5 days, then gradually decreased, suggesting reduced synthesis. On the basis of information in the literature it was anticipated that creatine loss would be 2% per day, in the absence of synthesis. The observed decrease in muscle creatine concentration was much less than was expected. Since the animals demonstrated considerable weight loss, it is possible that creatine was conserved from catabolized tissue or that the rate of creatine degradation was slowed. Extra-renal creatine synthesis could not be excluded.

In order to avoid longer periods of post-nephrectomy maintenance, C^{14}-tagged glycine was injected into the rats and the tagged creatine recovered as potassium creatinine picrate and counted. The studies indicated that under these conditions, the rate of creatine synthesis in nephrectomized rats was reduced to 5–18% of normal. Therefore, it appears that at least some creatine can be synthesized by a nonrenal pathway, possibly by the pancreas as recently suggested by Walker.

Studies on Calcium Phosphate Solubility in Urine

By *James S. Elliot.* Poliomyelitis Respiratory and Rehabilitation Center, Fairmont Hospital, San Leandro, California; Division of Urology, Department of Surgery, University of California School of Medicine, San Francisco; and Department of Medicine, Stanford University School of Medicine, San Francisco.

Available clinical and laboratory data indicate that renal calculus formation involves crystallization of salts from urine. Since crystallization will not occur unless a solubility product (or its equivalent) is exceeded, proper medical

management of the patient prone to develop calculi composed of calcium phosphate depends on quantitative data regarding calcium phosphate solubility in urine.

Urine specimens have been saturated with calcium phosphate by the addition of sodium phosphate or sodium hydroxide, incubated at 38 C. for one week, filtered, the solid phase identified by petrographic and x-ray diffraction analysis, the pH and the concentrations of calcium and phosphorus determined in the filtrate. When these data are plotted using Hodges' empirical equation, $pH = -2 \log (Ca) + \log (P) + K$, a straight line relationship is observed up to pH 7.0 whether the solid phase is composed of brushite or apatite. Calculation of an equation for the regression line makes possible the construction of a nomogram relating the 3 values, pH, (Ca) and (P) in urine saturated with calcium phosphate. If 2 of the values are known, the 3rd may be easily obtained from the nomogram. If all 3 values are known, it is possible readily to determine whether a given specimen of urine is over- or under-saturated with calcium phosphate.

Hypovolemic Shock and Hypotension in the Nephrotic Syndrome

By *Hiroshi Yamauchi and James Hopper, Jr.* Department of Medicine, University of California, School of Medicine, San Francisco.

The association of hypoalbuminemia and a low blood volume in nephrotics is well known. In spite of this well-known finding in nephrosis, clinical reports relating signs and symptoms of circulatory insufficiency are few. We are not aware of any studies of hypotension or shock. Our report deals with nephrotics in whom hypotension was observed.

Clinical and laboratory observations were made on 7 nephrotics. Blood volume measurements were made by use of radioactive chromium cell tag, serum protein determinations by the biuret method and serum albumin by sodium sulfite precipitation. Renal function was determined by the endogenous creatinine clearance.

The following results were obtained: Hypotension was observed in 5 nephrotics in the natural course of their illness. In 2, the hypotension was fatal. In 2 other cases, hypotension, shock and death were observed following surgical procedures (thoracotomy and renal biopsy). In the 4 cases of death, autopsies did not reveal any immediate, discernible cause of death other than effects of hypovolemia.

The following conclusions appear justified: (1) Shock secondary to hypovolemia may be an unrecognized mode of death in nephrotics. (2) Recognition of hypovolemia is essential if accidents are to be prevented. (3) Blood volume measurement is important in evaluation of a nephrotic.

NEOPLASTIC DISEASE

Increased Amount of Iodide in the Blood of Patients with Malignancy

By *W. A. Reilly, K. G. Scott and Gilbert L. Searle.* Radioisotope Service, V. A. Hospital, San Francisco, and Department of Radiology, University of California Medical School, San Francisco.

It was previously reported by one of us (K.G.S.) that in animals with transmissible tumors the total amount of blood inorganic iodides, but not protein-bound iodine, was markedly increased. The purpose of the present report is to compare these results with those of humans with malignancy. The blood iodide levels, after the ingestion of a small dose of I^{131}, of 49 normal patients have been compared to those of 49 patients who had various types of malignancy. The collected plasma samples were measured for I^{131} as iodide, PBI I^{131} and total I^{131}, using an anionic exchange resin. The group of malignant patients had statistically significant increases of inorganic iodide I^{131} and the total iodine counts, but normal PBI I^{131} at 24 and 48 hours after ingestion of I^{131}. The more extensive the metastases, the more the counts were increased. When done chemically, determinations of PBI were usually normal. In animal malignancy there is an increased destruction of mast cells with the release of histamine and 5-hydroxytryptamine, which suppresses urinary excretion of iodide. One wonders whether this also occurs in man. It is suggested that in human malignancy there is an increased amount of iodide in the blood.

Antitumor Activity of Reserpine

By *John B. Field, Annie Mireles, Edward C.*

Dolendo and Milton C. Kloetzel. Departments of Medicine and Chemistry, University of Southern California, and Los Angeles County General Hospital, Los Angeles.

After Goldin et al. pointed out the antileukemic effect of reserpine, further studies in other tumor systems were undertaken in these laboratories. A variety of reserpine, promazine (phenothiazines) and other pharmacologic depressants and tranquilizers have been evaluated. Marked inhibition of the growth of Sarcoma 180 and RC breast adenocarcinoma in mice has been induced with minute doses of reserpine and lesser inhibition, by rescinnamine, raunormine and several esters of reserpic acid. The promazines have had no antitumor activity. Although considerable weight loss occurred in the mice with the tumor inhibition, there did not appear to be a direct correlation between the two factors.

Within the limitation of the relatively few compounds studied, it would appear that the acyloxy group in position 3 of the quinolizine nucleus is required for antitumor activity. However, the nature of the aryl moiety within the acyloxy group is of some significance. For example, compounds which have shown antitumor activity have been esters of benzoic acid, 3,4,5-trimethoxybenzoic acid, 3,4,5-trimethoxycinnamic acid, 2-methyl-4-n-propoxybenzoic acid and 2,6-dimethyl-4-n-propoxybenzoic acid. Reserpine, the ester of 3,4,5-trimethoxybenzoic acid has been considerably more effective than any of the other agents examined to date. It would appear that a specific chemical configuration is associated with the antitumor effect of reserpine compounds and that the mild accompanying inanition is probably of no relevance.

The Quantitative Evaluation of Radiation Sensitizers by Means of the Puck Tissue Culture Technic

By *Malcolm A. Bagshaw.* Stanford Hospital, San Francisco.

It was the purpose of this series of studies to demonstrate that mammalian cancer cells might be rendered more sensitive to therapeutic irradiation by means of pretreatment with certain chemotherapeutic agents.

In general, known numbers of single HeLa cells were plated into Petri dishes. The dishes were randomized into 4 groups. Group A was incubated as an untreated control. Group B was treated with a known quantity of ionizing radiation. Group C was treated with a predetermined concentration of the sensitizing agent. Group D was treated with both the sensitizing agent and irradiation. Each group was incubated for 2 weeks, at the end of which time the surviving cells had multiplied to form macroscopic clones. The treated groups were compared with the control in order to determine the proportion of cells surviving.

The potentiation of radiation by chloroquine was equivocal, whereas potentiation by azaserine and DON (6-diazo-5-oxo-L-norleucine) was clearly additive and possibly synergistic.

It was concluded from these preliminary studies that the method permits a quantitative estimate of the efficacy of combined therapy with chemotherapeutic agents and x-ray. Further studies are to be carried out in order to confirm the action of the agents described above and to extend this research into a comprehensive investigation of other compounds.

RESPIRATORY SYSTEM

Methionine Turnover by Rat Tracheal Epithelium in Vitro and in Vivo

By *T. Timothy Crocker and Stefan R. Pelc.* Strangeways Research Laboratory, Cambridge, and King's College, London, England.

The purpose of these studies was to learn how well the functional and histologic integrity of respiratory tract tissue could be maintained in "organ culture."

The culture method of Fell and Mellanby was used and consisted of placing 2 mg., flat, segments of tracheal wall upon rayon squares which lay upon a clot made from rooster plasma and chick embryo extract.

The tissues in culture were fed S^{35} methionine for 24 hours, washed free of label and placed on fresh culture media. Cultures were fixed at 4 and 24 hours, and at 3, 7 and 11 days after labelling. Intact rats were inoculated with the same labelled compound and tracheal tissues fixed at the same time intervals. All tissues were sectioned at 5 μ and autoradiographs were made by the stripping-film technic.

Epithelial migration in cultured pieces was moderate, resembling wound healing. Migratory cells lost their original differentiation as ciliated and mucous cells. Counts of silver grains in the photographic films were made over standard microscopic areas of epithelium from cultured and from intact tracheas. Loss of radioisotope was parallel in epithelium of cultured treachea and in normal rat trachea, with a biologic half life for methionine of 5 days.

It is concluded that this mode of in vitro cultivation maintains differentiation of epithelium within physiologic limits and sustains protein metabolism at normal rates for the period of these studies.

Analog Computer Analysis of Flow Characteristics and Volume of the Pulmonary Vascular Bed

By *Daniel Parrish, Daniel T. Hayden, Wayne Garrett and Rex L. Huff.* Radioisotope Service, V. A. Hospital, and Department of Medicine, University of Washington, Seattle.

Using radioisotope dilution data obtained from the pulmonary artery and pulmonary vein of catheterized dogs, an analysis for pulmonary transfer characteristics was performed with an electric analog computer. The analog for these studies consisted of a cascade of linear delay units arranged so that both transport delay and dispersion could be independently varied. This technic makes it possible to characterize the pulmonary vascular bed without making any assumptions as to its nature. The results indicate that the lung does not act as a mixing pool, as suggested by Newman and others, but rather is a linear (i.e., laminar) flow system. In addition, it has been shown that an exponential extrapolation of the downslopes of the indicator dilution curves to calculate pulmonary blood volume by the Hamilton method results in a measurable error. The average pulmonary blood volume in 33 experiments was 11.7% of the total blood volume.

The Ventilatory Response of SCUBA Divers to CO_2 Inhalations

By *Herman F. Froeb.* Scripps Clinic and Research Foundation, La Jolla, California.

The ventilatory response of divers who work at increased atmospheric levels of carbon dioxide is impaired. The reason for this is either a change in the respiratory center response to the CO_2 - H^+ ion stimulus or to other phenomena not yet identified.

Sixteen male professional SCUBA (self contained underwater breathing apparatus) divers and 16 male nondivers (normals) were studied with respect to various respiratory physiologic parameters. The subjects were exposed to inhalations of carbon dioxide varying from 1 to 5% at both rest and exercise (2 miles per hour).

The divers and normals had comparable increases in minute ventilation at both rest and exercise until the 5% level of CO_2 inhalation was reached. Here the divers had a smaller rise in minute ventilation (due to slower rate, not smaller tidal volume) at both rest and exercise, although the mean expiratory pCO_2 (as an index of alveolar pCO_2) was the same for both groups (45 mm. Hg).

The data were then rearranged according to low and high ventilatory response to CO_2 inhalations irrespective of occupation (low group, 9 divers, 4 normals; high group, 7 divers, 12 normals). The pattern of response was similar enough to the previous diver and nondiver analysis to conclude that the difference in minute ventilation response to 5% CO_2 is a matter of individual variation and unrelated to occupation. At rest, the respiratory centers of the diver and those of the low ventilatory group are less sensitive to the CO_2 stimulus.

The Influence of Mechanical Factors on the Respiratory Work and Ventilatory Responses to Carbon Dioxide

By *Frederic Eldridge and John Davis.* Department of Medicine, Stanford University School of Medicine.

It has generally been considered that the ventilatory response to inspired CO_2 is an index of the sensitivity of the respiratory center, and that the low ventilatory response to CO_2 in patients with pulmonary disease is due to impaired sensitivity of the center, although several reported studies have implicated mechanical factors. This study was designed to show the effect of mechanical factors upon the respiratory work and ventilatory responses to CO_2. It was conducted in two parts.

1. Ventilation, alveolar pCO_2, and mechanical work of breathing were measured in 3 normal subjects during air breathing without added airway resistance and with 3 separate graded resistances inserted into the airway. Similar deter-

minations were made during the breathing of 2%, 4% and 6% CO_2 in air. During the breathing of air and 2% CO_2, increasing resistance led to a rise in alveolar pCO_2 and work of breathing, while ventilation remained constant. With higher CO_2 concentrations, increasing resistance led to sharp rises in pCO_2 and work, while ventilation actually decreased.

2. Ventilation and alveolar pCO_2 were determined in 10 normal subjects without added airway resistance, using 5% to 6% CO_2 as stimulus. Ventilation was then determined while breathing through 3 graded airway resistances, the alveolar pCO_2 being maintained at the same level as on the initial run without resistance. In the presence of a constant alveolar pCO_2 stimulus, increasing airway resistance caused a progressive drop in ventilation.

This study indicates that the work of breathing is an important parameter of respiratory stimulation by CO_2, whereas ventilatory response varies with the mechanical properties of the system. It is concluded that a low ventilatory response to CO_2 is not in itself indicative of respiratory center insensitivity.

A Comparison of Pulmonary Physiologic Studies with Gough Sections of Postmortem Lungs

By *Jerome E. Cohn, Terence H. Cochran and Charles T. Pinney.*

Gough has perfected a technic for studying excised or postmortem lungs fixed in inflation. The inflated lung is embedded in gelatin and coronal sections of the entire lung are cut, mounted and evaluated. Gough and MacLean, by studying the morphology of inflated lungs, have improved understanding of pulmonary pathology, especially pulmonary emphysema.

This report concerns a comparison of premortem studies, clinical and physiologic, with the postmortem findings in material handled by Gough's method.

First, a group of patients with pulmonary emphysema were evaluated. In all, the maximum breathing capacity was low; the ratios of volume to total lung capacity were elevated; there was marked obstruction to expiratory flow and abnormal intrapulmonary mixing of inspired gases. Arterial oxyhemoglobin desaturation was present in varying degrees, and in some there was hypercapnia. Each postmortem lung exhibited generalized centrilobular emphysema. Extensive coalescent emphysematous areas, as well as large bullae, often occurred. Pulmonary emboli were not uncommon. One subject, clinically indistinguishable from the others, had diffuse, severe, acute and chronic bronchitis, but no emphysema.

Another group was composed of 2 patients with nonemphysematous pulmonary disease. Both patients had alveolo-capillary block syndromes. Pulmonary function evaluation showed excellent ventilation, no significant obstruction to air flow, abnormal intrapulmonary mixing and severe diffusion impairment. Postmortem studies revealed extensive distortion of lung parenchyma by fibrosis, but no diffuse centrilobular emphysema.

Thus, good correlation between premortem and postmortem studies was found. This contrasts with most previous analyses and provides further evidence of the value of these pathologic technics.

Clinical Syndromes Associated with Deficient Lung Fibrinolytic Activity: A New Concept of Hyaline Membrane Disease and Cystic Fibrosis of the Pancreas

By *Jack Lieberman.*

Diseases in which excessive fibrinous or proteinaceous material is deposited within parenchyma, ducts or chambers of an organ could be explained by a defect in liquefaction capabilities of these tissues, perhaps due to a deficiency in the tissue activator of plasminogen (T.A.P.). Two such conditions are hyaline membrane disease of the newborn and cystic fibrosis of the pancreas.

Lung and several other organs were studied for T.A.P. activity by a modification of the fibrin-plate method of Astrup and Mullertz. Six children with cystic fibrosis and 49 infants and fetuses dying perinatally were studied. The latter included 8 with pulmonary hyaline membrane formation. T.A.P. was found in lung as early as the 3rd month of gestation. Thirty-three of 41 specimens without pulmonary hyaline membrane formation showed lung T.A.P. activity. None of 8 infants with hyaline membranes nor any of 6 children with cystic fibrosis showed this activity. Guinea pigs and rabbits, subject to hyaline membrane formation from oxygen poisoning, demonstrated this same deficiency of lung T.A.P. in contrast to rats whose lungs contain the enzyme and are more resistant to formation of the membrane.

These data suggest that a predisposing factor in hyaline membrane formation of the newborn is the absence of T.A.P. activity in lung.

An interaction between amniotic fluid- or tissue-thromboplastin and other clotting factors in an alveolar effusion results in deposits of fibrin which are transformed into hyaline membrane postnatally. T.A.P. is postulated to function in the lysis of these fibrin deposits. The defect is not related to prematurity, but may represent a genetic aberration.

In cystic fibrosis a deficiency in lung T.A.P. plus a similar defect in the exocrine portion of the pancreas might result in deranged mucoprotein synthesis.

It is possible that defective tissue fibrinolytic activity will be found to be associated with other disease states. A new concept of "afibrinolytic" disease may thus evolve.

PROGRAM, SOUTHERN SECTION

American Federation for Clinical Research

Thursday and Saturday, January 22 and 24, 1959
Jung Hotel, New Orleans, Louisiana

Dr. Ellard M. Yow, Presiding

Presentations will be limited to ten minutes

9:00 A.M.

1. **The Ear Lobe Phagocytic Monocyte in Bacterial Endocarditis.**
   *Lamar Crevasse,** Gainesville, Florida. (Introduced by *Samuel P. Martin.***)
   page 160
2. **Epidemiologic Studies of an Outbreak of Staphylococcal Disease.**
   *Harris D. Riley, Jr. and William Kilgore,** Oklahoma City. *page 159*
3. **The Effect of Bacterial Endotoxin on Adrenal Medullary Function.**
   Richard H. Egdahl, Richmond, Virginia. *page 158*
4. **Studies on Plasma Cell Leukemia.**
   *William J. Hammack and Walter B. Frommeyer, Jr.,*** Birmingham, Alabama. *page 133*
5. **A Reticulocyte Response Following the Administration of Serine to Patients with Tropical Sprue in Relapse.**
   C. E. Butterworth, Jr. and Enrique Perez-Santiago,** Birmingham, Alabama and San Juan, Puerto Rico. (Introduced by *Walter B. Frommeyer, Jr.***) *page 156*
6. **Gall Bladder Mucoproteins in Cholelithiasis.**
   Robert B. Giles, Jr., Dallas, Texas. *page 153*
7. **Chemical and Enzymatic Studies of Normal and Diseased Human Liver.**
   *John T. Sessions, Jr., W. Geoffrey Wysor, Jr.,** Nathan A. Womack* and Oscar L. Sapp, III,* Chapel Hill, North Carolina. *page 153*

INTERMISSION

8. **Cerebrospinal Fluid in Jaundiced Patients.**
   *John T. Galambos and Donald G. Rosenberg,** Atlanta, Georgia. *page 168*
9. **Circulating Anti-Human Kidney Antibodies in Renal Disease.**
   Norman C. Kramer, Mary F. Watt and Alvin E. Parrish,* Washington, D. C. *page 164*
10. **The Influence of Various Diuretic Agents on the Urinary Excretion of Magnesium in Non-Edematous Subjects.**
    William O. Smith, Adrian Kyriakopoulos, David C. Mock* and James F. Hammarsten,* Oklahoma City. *page 162*
11. **Alterations in Renal Hemodynamics during Surgical Resection of Abdominal Aortic Aneurysms.**
    *William B. Berry,** George C. Morris, Jr. and Michael E. De Bakey,* Houston, Texas. *page 163*
12. **Comparison of the Cardiac Output Responses to Hyperventilation and Exercise.**
    Howard K. Thompson, Joseph N. Berry* and Henry D. McIntosh,* Durham, North Carolina. *page 136*

BUSINESS MEETING

1:30 P.M.

Dr. John C. Rose, Presiding

13. **An Experimental Appraisal of Heparin in Burns.**
    Byron E. Green and Curtis P. Artz,*** Jackson, Mississippi. *page 169*
14. **The Effects of Lung Elastin and Surface Forces on the Physical Properties of the Lungs.**
    Bill F. Hefley and John A. Pierce,* Little Rock, Arkansas. *page 170*
15. **The Role of Hypercapnia in the Control of Cardiac Output.**
    *David W. Richardson, A. J. Wasserman,**

*By Invitation
**Senior Member

*W. S. Dingledine and J. L. Patterson, Jr.,*** Richmond, Virginia. *page 136*

16. **The Effect of Parenterally Administered Vitamin B_{12} on Muscular Contraction in Myxedema.**
*John D. Lawson and Arthur S. Weissbein,** Fort Sam Houston, Texas. *page 143*

17. **The Effect of Endogenous Insulin Secretion upon the Magnitude of Hepatic Binding of Labeled Insulin during a Single Transhepatic Circulation in Human Subjects.**
*Norman Kaplan** and *Leonard L. Madison,* Dallas, Texas. *page 145*

18. **Effects of Thyroid Hormone on the Maturation of the Central Nervous System.**
*Max Hamburgh,** New York. (Introduced by *Edna N. Sobel.*) *page 142*

19. **Synthesis of Cholesterol and Fatty Acids in Peripheral Nerve.**
*Ann H. Hughes** and *Sven G. Eliasson,* Dallas, Texas. *page 167*

20. **The Effect of Denervation on the Glucose Uptake and Insulin Sensitivity of the Isolated Rat Diaphragm.**
*John Buse and Maria Gordon Buse,** Charleston, South Carolina. *page 146*

21. **Irreversible Myxedema, With and Without Coma.**
*John F. Nickerson,** *Samuel B. Barker,*** *Jean H. McNeil** and *S. Richardson Hill, Jr.,* Birmingham, Alabama. *page 142*

22. **In Vitro Synthesis of Lipids from C-14 Acetate by Adipose Tissue from 4 Types of Obese Mice.**
*Guy Hollifield, William Parson*** and *Carlos R. Ayers,** Charlottesville, Virginia. *page 149*

23. **The Renal Clearance of Endogenous Free and Conjugated 17-Hydroxycorticosteroids in Normal and Hypertensive Subjects.**
*Ludwig Kornel,** Birmingham, Alabama. (Introduced by *S. Richardson Hill, Jr.*) *page 147*

24. **Effect of Chronic Hypercalciuria on Renal Conservation of Sodium and Water.**
*John R. Gill, Jr.** and *Frederic C. Bartter,*** Bethesda, Maryland. *page 161*

Joint Program with Southern Society for Clinical Research

SATURDAY, JANUARY 24

25. **Estrogen and Progesterone Levels in Fetal and Maternal Plasma at Parturition.**
*John R. K. Preedy, Elsie H. Aitken,** *Bruce Eton** and *Roger V. Short,** Atlanta, Georgia. *page 157*

26. **Ferrokinetics and Erythrokinetics in Sarcoidosis.**
*Norman H. Bell,** *Martin E. Liebling** and *James M. Stengle,** Bethesda, Maryland. (Introduced by *John P. Utz.*) *page 132*

27. **Intravenous Fibrinolysin Therapy of Induced Radio-Opaque Coronary Thrombi.**
*Paul W. Boyles, William H. Mayer** and *Marvin B. Slotkin,** Coral Gables, Florida. *page 137*

28. **Adrenocortical Function after Long-Term Corticoid Therapy.**
*Gabriel G. Carreon,** *John J. Canary and Laurence H. Kyle,*** Washington, D. C. *page 146*

29. **Lung Volume Changes with Extreme Obesity.**
*James K. Alexander, Aubrey E. Guthrie,** *R. Sakaguchi,** *H. Wayne Crawford** and *V. William Cole,** Houston, Texas. *page 171*

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BLOOD

Ferrokinetics and Erythrokinetics in Sarcoidosis

By *Norman H. Bell, Martin E. Liebling and James M. Stengle.* Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases, and General Medicine Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

The mechanism of anemia in sarcoidosis has not been clearly elucidated. Twenty-five randomly selected sarcoidosis patients in whom there was no clinical evidence of hemolysis, blood loss or infection and to whom no therapy was being given were studied. Serial iron determinations over a 24-hour period revealed significantly lower values than those found in normal individuals. Total iron-binding capacities (TIBC's) were normal. The low serum iron values were apparently not related to the severity or duration of disease. Six patients were studied further with Cr^{51} for RBC survival and with Fe^{59} for iron turnover rate. Bone marrow aspirates were morphologically normal. Hematocrit determinations in 4 of the 6 were 38% or below. All but one case had normal RBC survival. In this patient the survival was slightly shortened. This was the only case studied with a history of splenomegaly and was one of two with a rapid plasma iron disappearance. No defect was demonstrable in bone marrow uptake of Fe^{59} or in the quantitative appearance of iron in RBC. Excessive storage of Fe^{59} in the liver or spleen was not detectable by in vivo counting.

Blood Volume Studies in the Hypothermic Dog Employing Cr^{51}-labeled Erythrocytes

By *Delford L. Stickel, George S. Eadie, W. Glenn Young, Jr. and Will C. Sealy.* Departments of Surgery and Physiology, Duke University Medical Center, Durham, North Carolina.

Reported studies of the effect of hypothermia on the circulating blood volume have shown variable results. The purpose of this study is to explore the subject further and to include observations of the mixing curves of labeled RBC injected into the circulation, inasmuch as knowledge of mixing time is of critical importance in the determination of blood volume in a given physiologic state. Nine previously splenectomized mongrel dogs under intravenous Nembutal anesthesia were cooled to 28 C. Mixing curves of injected Cr^{51}-labeled RBC were recorded, from which mixing time and circulating RBC volume were calculated. Immediately preceding cooling (Group I) or after rewarming (Group II), con-

trol observations were made at normal temperature. Mixing time (95% of complete mixing) averaged 12 minutes at normal temperature and over 20 minutes during hypothermia. No significant changes occurred in circulating RBC volume. "Fast" and "slow" circulating RBC volumes (Fed. Proc. *15*:53, 1956) and rates of transfer between these components were estimated. These findings explain in part previous reports of changes in blood volume and circulatory dynamics occurring during hypothermia.

It is concluded that although hypothermia alters the mixing curve and the distribution of injected labeled erythrocytes there is no change in total RBC volume.

Studies on Plasma Cell Leukemia

By *William J. Hammack and Walter B. Frommeyer, Jr.* Department of Medicine, Medical College of Alabama, Birmingham, Alabama.

The relationship of plasma cell leukemia to multiple myeloma and other leukemias is not clear. The purpose of this study was to investigate the clinical and laboratory manifestations of 4 patients with the diagnosis of plasma cell leukemia.

Plasma cell morphology of the peripheral blood and bone marrow is reviewed. Paper electrophoretic patterns of the serum and urine were done by Spinco Model RB system. Sedimentation characteristics of the serum proteins were studied by Spinco Model E Analytical Ultracentrifuge, and immunologic studies were made with the Ouchterlony modification of the gel diffusion technic.

Three patients had a rapidly fulminating course with evidence of a bleeding tendency. One patient has had a remarkable clinical remission; however, some immature plasma cells can still be found. Electrophoresis revealed a tall, narrow gamma globulin peak in the sera of 3 patients, while one patient's serum had only minor abnormalities. Electrophoresis of the urine revealed a protein having a mobility identical to the serum peak. The serum of 2 patients reacted with antimacroglobulin antiserum, and on ultracentrifuge study an elevated macroglobulin was demonstrated. One of the macroglobulins was also a cryoglobulin. Cell morphology varied widely, but in general the cells were more immature than in myeloma and resembled reticulum cells or lymph stromal cells.

It is concluded from this study that clinically plasma cell leukemia resembles an acute leukemia and usually terminates more rapidly than myeloma. Protein abnormalities occurred less commonly than in myeloma, and in 2 instances these were macroglobulins instead of the usual myeloma proteins.

BLOOD PROTEINS

The Metabolism of I^{131}-labeled Bence-Jones Protein

By *William H. Perkins, James E. Doherty and Eugene J. Towbin.* Department of Medicine, University of Arkansas School of Medicine, and Radioisotope Service, V. A. Hospital, Little Rock, Arkansas.

Past studies of Bence-Jones protein metabolism have been concerned mostly with its renal excretion. We have undertaken to determine to what extent it is removed from the circulation by metabolic degradation rather than by renal excretion.

After concentration from urine by cold acetone precipitation, the Bence-Jones protein was separated from other urinary proteins by curtain electrophoresis. It was labeled with I^{131}. These procedures did not change its electrophoretic properties.

The labeled protein was injected intravenously into 2 patients without multiple myeloma and with normal renal function and into 1 patient with multiple myeloma. Serial urine samples and blood samples were collected from all the patients.

After paper strip electrophoresis, the serum samples of all the patients showed a zone radioactivity between the beta and gamma globulins. Plasma was added to urine samples as a carrier, and all proteins were precipitated with cold acetone. After dissolving in normal saline, the urinary protein from all the patients was found, on paper electrophoresis, to contain a protein lying between beta and gamma globulin.

Protein-bound iodine determinations were made on all serum and urine samples. Turnover rates showed a fast and a slow component. The fast component had a half life of about 900 minutes and the slow component about 3,000

minutes in all patients. The renal plasma clearance of Bence-Jones protein was found to be about 1.0 cc./min.

Approximately 80% of the injected Bence-Jones protein was metabolized by degradation and 20% by renal excretion in all patients.

Cryofibrinogenemia: a Physico-Chemical Study

By *Michael Lory Campbell, William J. Hammack and Walter B. Frommeyer, Jr.* Department of Medicine, Medical College of Alabama, Birmingham, Alabama.

The purpose of this study is to characterize a cold precipitable protein manifested clinically by purpura and occlusive vascular phenomena and unexplicable in terms of an associated disease.

Double oxalate was used as an anticoagulant for plasma. Wintrobe tubes were employed for cryocrits. Blood proteins were determined by the Spinco Model RB paper electrophoresis system and Model E Analytical Ultracentrifuge. Glendening-Olson-Page method for quantitative fibrinogen was used. Coagulation factors were assessed using standard procedures.

Prothrombin consumption and coagulation, prothrombin and recalcification times of the patient's blood and plasma at 37 C. were normal. Plasma electrophoresis showed a heavy homogeneous peak with the mobility of fibrinogen which correlated well with the hyperfibrinogenemia of 0.889 Gm. %. Storage of plasma and serum overnight at 4 C. caused precipitate to appear only in the plasma, the cryocrit being 21 mm. after cold centrifugation. Supernatant plasma prothrombin time was 99 seconds, while equal aliquots of supernatant and barium sulfate-treated normal plasma corrected this to only 25 seconds. Supernatant plasma recalcification time was infinite. Electrophoresis of the supernatant showed a decrease in the fibrinogen peak, this correlating well with the observed 25% reduction in quantitative fibrinogen. Electrophoresis of the precipitate in normal saline showed a component only at point of origin. Addition of thrombin to this solution resulted in a firm clot, and electrophoresis of this supernatant showed no evidence of fibrinogen. Plasma ultracentrifuge patterns were normal, and no macroproteins were present.

It is concluded that the purpura and vascular occlusive phenomena observed in this patient were due to a cryoprotein having the physico-chemical properties of fibrinogen. A qualitative defect of this fibrinogen, adsorption of certain coagulation factors on the cryofibrinogen and intravascular precipitation of this protein may play a cardinal role in the pathophysiology.

BONE

Effect of Cobalt Radiation on Healing of Tooth Socket Following Extraction and Therapy

By *J. Harold Conn, William R. Fain, Robert D. Sloan and Gerald W. Farrell.* Departments of Surgery, Radiology and Dentistry, V. A. Center, and University of Mississippi Medical Center, Jackson, Mississippi.

Osteoradionecrosis of the maxilla or mandible is the most common complication of radiation therapy of intra-oral neoplasia. This is an extremely painful and disabling condition which can be avoided only by extraction of teeth in the irradiated area prior to treatment. In cases of rapidly growing intra-oral malignancy, how soon can radiation therapy be started following tooth extraction without risk of non-healing or osteoradionecrosis of the exposed tooth socket? The following experiment was devised to examine this question.

Teeth were extracted from 20 dogs, divided equally into control and irradiated groups. The treated group received therapeutic doses of cobalt to the extraction area. Therapy was begun on the 2nd to 4th post-extraction day and 500 r given daily for a total of 4,500 r. This is the usual therapeutic dose for intra-oral malignancy. Length of healing time was determined both grossly and histologically.

All of the control animals healed completely in an average time of 16.5 days. Eight of the irradiated animals healed in an average time of 44 days. The other 3 dogs in the treatment group died unhealed in an average of 32 days.

The results indicated that, although immediate irradiation does cause a significant delay in wound healing, the gingiva eventually healed without evidence of osteoradionecrosis. This is significant, for apparently radiation therapy can be started immediately on rapidly growing neo-

plasms without fear of wound-healing failure or osteoradionecrosis.

An Intravenous Calcium Retention Test in Bone Disease

By *Leo Lutwak and G. Donald Whedon*. Metabolic Diseases Branch, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland.

The utilization of calcium is frequently abnormal in metabolic diseases of bone. A simple calcium tolerance test described by Lichtwitz et al. has been evaluated in 70 studies on 35 individuals with and without radiologic evidence of bone disorders and compared with the standard 4-hour infusion test (Nordin and Fraser). A single dose of 176 mg. of calcium was administered intravenously and urine collected in 4-hour aliquots for 20 hours thereafter. The urinary excretion of calcium after the injection was compared with that for the same hours on the day prior to the test. Hospitalized subjects received constant diets the week prior to test days; outpatients were instructed in a constant diet which was consumed for 3 days before and on the days of the urine collections. Results have been obtained in 9 normal adults, as well as in patients with the following diagnoses: rheumatoid arthritis, osteoporosis, osteomalacia, osteitis deformans, osteogenesis imperfecta, hyperparathyroidism, myositis ossificans, acromegaly. The tests were performed at dietary intakes of 150 to 2400 mg. of calcium per day and, in some of the subjects, under the influence of corticosteroids, estrogens, androgens, triiodothyronine or vitamin D.

In osteomalacia and osteogenesis imperfecta, the retention of calcium was high; in osteoporosis it was within the normal range. Corticosteroids produced decreased retention, with increased retention 2 to 3 weeks after the steroid was discontinued. Increasing the dietary intake of calcium increased the retention of the intravenously administered dose in osteoporosis; this may be increased further by gonadal steroids.

The accumulated excretion of calcium in the 12 hours following the injection provides a better index of calcium utilization than in 24 hours. Thus, a relatively simple diagnostic test of calcium metabolism and the effects of therapy thereon may be based on a single injection of calcium and the collection of 12-hour urinary samples the day before and the day of the test while the subject is on a relatively constant diet.

CARDIOVASCULAR SYSTEM

The Interrelationship of Central Nervous System Arousal State and the Hemodynamic Effects of Epinephrine

By *J. Caulie Gunnells, Ralph J. Gorten, Morton D. Bogdonoff and James V. Warren*. Department of Medicine, Duke University School of Medicine, Durham, North Carolina.

The changes in cardiovascular function that accompany experimentally induced alterations in affect are similar to those associated with the infusion of epinephrine. The feeling of anxiety that is produced by situational stimuli and the subjective experiences attending epinephrine injection suggest the possibility that epinephrine might be the common mediator of both the affective and cardiovascular responses. Of further interest is whether central nervous system activity either augments or, in some way, conditions the cardiovascular changes. In an attempt to assay the contribution of central nervous system activity to the magnitude of cardiovascular responsivity, epinephrine has been administered to individuals who have also received meprobamate.

Cardiac output (determined by the dye dilution technic) was measured in 12 male subjects immediately following epinephrine (10 μg. i.v.) injection both before and one-half hour after receiving meprobamate (800 mg. i.v.). At this dosage level of meprobamate, no specific changes in cardiovascular function have been reported, but electroencephalographic effects can be detected. Following the meprobamate infusion, all subjects reported a feeling of relaxation, a decrease in perceptivity and awareness, and a diminution in the usually dramatic subjective effects of the epinephrine injection.

The cardiac output response to epinephrine following meprobamate was greater than the premeprobamate response in 6 of the 12 subjects. In 3 subjects, there was a decreased response, and in 3, no change. In attempting to evaluate the factors that might influence this variable effect of meprobamate upon epinephrine response,

it was noted that the 3 subjects with a decreased response were considered to be extremely anxious and aroused individuals.

We have concluded from these studies that the status of central nervous system arousal does appear to influence cardiovascular responsivity to epinephrine, but that this relationship is not uniform and may be paradoxical (decreased arousal, increased responsivity).

Comparison of the Cardiac Output Responses to Hyperventilation and Exercise

By *Howard K. Thompson, Joseph N. Berry and Henry D. McIntosh.* Department of Medicine, Duke Medical Center, Durham, North Carolina.

Studies in this laboratory designed to elucidate the hemodynamic response to hyperventilation suggested that increased heart rate and cardiac output observed during vigorous hyperventilation might be in part due to muscular exercise. The present study was designed to compare the hemodynamic changes accompanying hyperventilation and exercise. Fourteen recumbent males were studied: 7 during leg exercise, 7 during hyperventilation, (40/min.), both for 3 minutes. Cardiac output and "central blood volume" (dye-dilution technic), oxygen consumption, mean central venous pressure, arterial pressure and total peripheral resistance were measured.

Cardiac output and heart rate approximately doubled and peripheral resistance decreased by 50% during both stresses. Oxygen consumption increased approximately 2-fold with hyperventilation but 4-fold with exercise. Mean central venous pressure was unchanged during either maneuver. Arterial pressure increased during exercise but showed little change during hyperventilation. "Central blood volume" increased 42% with exercise ($P < 0.02$) but was unchanged with hyperventilation.

In an attempt to see whether the cardiac output response to these maneuvers could be modified, 2 mg. atropine sulfate was injected intravenously and hyperventilation and exercise repeated. Following atropinization, hyperventilation and exercise produced an additional and similar increase in heart rate.

The cardiac output response to hyperventilation was strikingly different before and after atropine. Although the cardiac output increased 100% with hyperventilation before atropinization, the increase over the resting non-atropinized control value was only 42% when hyperventilation and atropine were combined. In contrast, the 100% increase in output with exercise was unaffected by the addition of atropine. Arterial pressure and "central blood volume" again increased with exercise, but not with hyperventilation.

These studies indicate that although the cardiac output and heart rate increased equally with hyperventilation and exercise, only exercise caused an increased arterial pressure and "central blood volume." Atropine produced an unexpected lessening of the cardiac output rise during hyperventilation, but not during exercise. The data suggest that the cardiac output response to hyperventilation and exercise may be brought about by different mechanisms.

The Role of Hypercapnia in the Control of Cardiac Output

By *David W. Richardson, A. J. Wasserman, W. S. Dingledine and J. L. Patterson, Jr.* Medical Service, Veterans Hospital, and Department of Medicine, Medical College of Virginia, Richmond, Virginia.

Of physiologic factors important in the regulation of cardiac output, blood carbon dioxide tension (pCO_2) has been the subject of few studies, and in these the results have been contradictory. The present investigation reports marked change in hemodynamics associated with hypercapnia induced by breathing 7% CO_2 for 7 minutes in 16 experiments in 10 normal human volunteers. Cardiac output was estimated by peripheral injection of indocyanine green dye, arterial pCO_2 using the Riley bubble-equilibration technic, and respiratory rate and volume using a calibrated pneumotachograph. In 10 additional experiments, subjects performed maximal voluntary hyperventilation with a variable inspired CO_2 concentration, adjusted to maintain constancy of end-tidal pCO_2, the latter monitored with a Liston-Becker CO_2 analyzer.

During voluntary hyperventilation with alveolar (and arterial) pCO_2 maintained at control levels, the average respiratory minute volume of the group increased from 13 to 33 liters, and average cardiac index remained constant at 3.6 L. min.$^{-1}$ M.$^{-2}$. During breathing of 7% CO_2, there was increase in RMV from 9 to 45 liters, in pCO_2 from 42 to 58 mm. Hg ($p < .001$) and in cardiac index from 2.9 to 4.2 L./min./M.^{2} ($p < .001$). Mean arterial pressure rose from 89 to 105 mm. Hg ($p < .001$) during 7% CO_2 breathing, but rose only 3.5 mm. Hg ($p > .1$)

during vigorous breathing without change in pCO_2.

The reported findings demonstrate that the large alterations in circulatory dynamics associated with induced hypercapnia are the result of changes in blood CO_2 tension and not of the vigorous respiratory movements. Doubt is cast on the time-honored dogma of the thorax as a blood pump.

PR Segment Formation

By *Cesar A. Caceres, George A. Kelser, Jr. and W. Raymond Mize.* Department of Medicine, George Washington University Hospital, Washington, D.C.

The necessity for precision, and the possibility of its attainment, in the measurement of electrocardiographic waves is illustrated by measurements of the PR segment. Precise methods of measurement yield significant information not obtained by routine technics of interpretation.

Optical magnification of standard electrocardiograms and electronic amplification of electrocardiograms with cathode ray tube electrocardiographs was used.

In optically magnified standard electrocardiograms the terminal limb of the P wave appears to fuse with the initial deflection of the QRS, making it impossible to determine the existence of an interval between them, or suggesting an interval of less than 0.06 seconds between them.

In electronically amplified electrocardiograms the segment that follows the terminal limb of the P wave is usually present for less than 0.04 second before a deflection of the ventricular complex is apparent. The rapidity with which these relatively minor changes of potential occur suggests that mechanical limitations of direct writers, and limitations imposed by standard sensitivities in these and other instruments, prevent adequate visualization of electrocardiographic waves. These limitations account for the inscription of the conventional PR segment.

The close approximation of the terminal slope of the P wave and the beginning of the ventricular complex within a short interval suggests that the normal PR segment is derived from either the junction of electrical signals due to the termination of atrial depolarization and the onset of ventricular depolarization, or the algebraic summation of the simultaneous potential changes from these two events.

Intravenous Fibrinolysin Therapy of Induced Radio-Opaque Coronary Thrombi

By *Paul W. Boyles, William H. Mayer and Marvin B. Slotkin.* Coagulation Research Laboratory, Miami Heart Institute, Miami Beach, Florida, and Departments of Medicine and Surgery, University of Miami School of Medicine, Coral Gables, Florida.

Recent studies have demonstrated the effectiveness of intravenous fibrinolysin therapy in the treatment of intravascular blood clots. The present investigation illustrates the use of intravenous fibrinolysin therapy on induced coronary thrombi in dogs.

Utilizing a recently developed method, radio-opaque coronary thrombi were produced by the injection of a mixture of radio-opaque media and thromboplastin into a temporarily occluded coronary vessel. The fate of these clots was determined in both treated and control animals by two different methods. In one type of experiment, the clot was observed by serial x-ray examination which was correlated with necropsy data. In another type of experiment, the clot was observed directly in the intact coronary vessel through the open chest wall of the dog.

Human fibrinolysin was administered intravenously into the general systemic circulation by means of a superficial vein over a one-half hour period. Serial observations were recorded on the various coagulation factors, fibrinolytic activity and transaminase level. Serial electrocardiographic recordings were performed in most of the dogs, and careful dissection of the coronary vessels was performed after varying periods of observation.

In the series of dogs studied, no significant acceleration in the lysis of the induced radio-opaque coronary thrombi was noted despite the administration of fibrinolysin in doses sufficient to completely lyse the circulating fibrinogen of the dogs. Transaminase determinations during the first 6 hours after the induction of the coronary occlusion were similar in both the treated and control animals. The injection of fibrinolysin produced a shortening of their whole blood lysis time and an increase in the fibrinolytic activity, as measured by the synthetic substrate method of Sherry with TAME (p-toluenesulfonyl-l-arginine methyl ester).

The data demonstrate that intravenous fibrinolysin administered into the general circulation does not significantly accelerate the lysis of experimental coronary thrombi in dogs. This sug-

gests that segmental injection of fibrinolysin will be necessary to obtain significant in vivo clot lysis of thrombi in coronary arteries.

An Evaluation of Coronary Artery Disease with Paper Electrophoresis of Serum Proteins

By *Raymond J. Leffler.* East Tennessee Baptist Hospital, Knoxville, Tennessee.

The purpose of this study was to apply paper electrophoretic studies of lipoproteins to patients with coronary artery disease.

A durrum paper electrophoretic cell was used to hold paper strips which were then stained with Sudan black B and scanned with a densitometer (Analatrol, Spinco). An atherogenic index was calculated giving 1.75 greater weight to lipoprotein globulin larger than SF_4.

A graphic representation of these atherogenic indices in relation to age showed a highly significant difference in males with and without coronary artery disease. No such relationship was found in females who are the subject of further study. Non-coronary male patients had lower atherogenic indices, especially in the older age group. Males with coronary artery disease had atherogenic indices above a slanting line which was higher in the younger age group and lower in the older age group. Successive atherogenic indices in individual patients show that atherogenic indices can be reduced with low fat-unsaturated fat diets.

It is speculated that coronary artery disease is essentially a sex-linked constitutional defect of males in which fats are poorly utilized, comparable to diabetes mellitus in which carbohydrates are poorly utilized. This defect can be partially controlled by low fat-unsaturated fat diets.

Effect of Coronary Artery Ligation on Heart Weight

By *Tom D. Norman and Richard Coers.* Department of Pathology, University of Arkansas, Little Rock, and University of Mississippi, Jackson.

Whether occlusive coronary artery disease causes cardiac hypertrophy has challenged investigators for many years. The present investigation represents an attempt to answer this question by observations on rat hearts following coronary artery ligations.

Forty-six male Holtzman rats which lived after either ligation of an anterior descending branch of the left coronary artery or a comparable sham operation were studied. Eleven ligated and 11 sham-operated animals were killed 6 weeks after operation. (Twelve ligated and 12 sham-operated rats will be killed 12 weeks after operation.) The hearts were excised, opened, washed, blotted and weighed on an analytic balance; after excision of atria from ventricles and right ventricles from septa, right ventricles and left ventricles with septa were weighed.

Hearts of ligated rats killed 6 weeks postoperatively were grossly, although somewhat inconsistently, enlarged when an infarct was present (8 rats), as compared to the sham-operated control animals. Since final mean body weight of ligated rats (305 Gm.) was less than that of control animals (322 Gm.), a *t* test was used to study the difference in mean heart weights of ligated rats with infarcts and sham-operated animals. P was 0.05, considered to be statistically significant. The right ventricle was enlarged out of proportion to the left, as determined by gross observations and relative weights.

It is concluded that there was an increase in heart weight (presumed to be hypertrophy) in rats following ligation of a main coronary artery. Although this is not strictly comparable to the situation existing in man with occlusive coronary artery disease, it is believed to support strongly the thesis that such disease of coronary arteries may play a part in the development of cardiac hypertrophy.

The Effect of pH on the Rate of Coronary Perfusion and on the Cellular Sodium, Potassium and Water in the Isolated, Perfused Rat's Heart

By *Isaac M. Taylor and D. T. Young.* Department of Medicine, University of North Carolina School of Medicine, Chapel Hill, North Carolina.

Rats' hearts, kept alive and beating in vitro by perfusion of the coronary system via the aorta, have been used to study the effect of changes in pH of the perfusion medium upon the rate of coronary flow and upon intracellular sodium, potassium and water. Decreasing pH to 7.0 by increasing the pCO_2 of the perfusion medium or by adding HCL to the perfusion medium produces a marked decrease in coronary flow and an increase in intracellular water. Intracellular sodium appears to increase. Intracellular potassium content is not much changed, but the concentration of potassium falls as a result of the

increased intracellular water. Concommitantly, there is diminished force of contraction of the heart muscle and slowing of the heart rate. The magnitude of these chemical and mechanical changes appears to vary with the degree of acidosis induced. The changes are reversed by restoration of a normal pH. Alkalosis speeds coronary perfusion and increases the vigor of the isolated heart's contraction; its effect upon tissue water and cations is under study.

Clearance of Intraarterial RISA: a New Method for the Separate Estimation of Skin and Muscle Blood Flow

By *Edward D. Frohlich, Frank J. Fedor and Edward D. Freis*, Mt. Alto V. A. Hospital, and Department of Medicine, Georgetown University School of Medicine, Washington, D. C.

Previous studies from this laboratory suggest that calf and foot blood flow may be individually estimated following intrafemoral arterial injection of I^{131}-labelled albumin. Scintillation probes attached to recorders are placed separately over calf and sole. The present study was undertaken to determine which of the following parameters, appearance time, peak height, clearance (downslope gradient) or area, provides the best index of blood flow.

Young subjects without known circulatory disease were studied in a cold room. Isolated muscle hyperemia was produced either by local exercise of calf muscles or by intraarterial Arlidin. Skin or foot hyperemia was produced by intraarterial Priscoline or intravenous hexamethonium. The latter drugs produced significant increases in foot but not calf skin temperatures.

Change in the rate of clearance followed the expected hemodynamic changes in every instance. In the foot, clearance was unchanged after exercise or Arlidin, but increased markedly after hexamethonium or Priscoline. In the calf, local exercise and Arlidin produced the greater increases in clearance.

The other parameters were unreliable since they depend, in addition, upon relative hyperemia in other areas of the leg which can divert and dilute the isotope. For example, Arlidin dilated thigh as well as calf muscles and, therefore, induced variable changes in peak height and area over the calf. Likewise, calf exercise shortened appearance time in the foot in the absence of significant hyperemia of the foot.

These studies suggest that clearance of intraarterial RISA provides a new and simplified method for the separate and simultaneous determination of skin and muscle blood flow in the human lower extremity.

Increased Activity of the Plasma Permeability Factor in Familial Hereditary Angio-Edema

By *Nathaniel S. Landerman, Elmer L. Becker, Harold E. Ratcliffe, Michael J. Davis and Edward J. Kamin*. Department of Medicine, Walter Reed Army Hospital, and Departments of Immunochemistry and Dermatology, Walter Reed Army Institute of Research, Washington, D. C.

Miles and co-workers discovered and characterized a powerful capillary permeability–increasing agent, the Plasma Permeability Factor (P.F.) or PF/dil, in the plasma of normal animals and man. The possibility that increased P.F. activity might be involved in the disordered vascular permeability of familial hereditary angioedema led us to assay for P.F. in such a case and in 7 normal controls.

An in vivo P.F. assay technic similar to that reported by Stewart and Bliss in normal man was used. Each subject was given 50 mg. of T-1824 intravenously. Then 0.1 ml. volumes of undiluted and serially diluted autologous plasma were injected intradermally. The diameter of the blue wheal at each injection site was recorded 30 minutes later as an index of P. F. activity.

The patient's P. F. activity was invariably and significantly higher than that of the 7 normal controls. In addition, when tested in the patient and 1 control, the patient's response was greater to her own plasma than to the control's plasma, whereas the control's response was greater to the patient's plasma. P. F. activity was inhibited in the patient and a control by crystalline soya bean trypsin inhibitor, but not by Neo-Antergan or Prostigmin. Benadryl did not alter a control's P. F. activity, but did inhibit the patient's P. F. activity in the higher dilutions. Studies in the patient concerning other endogenous capillary permeability–increasing agents (histamine, acetylcholine, serotonin and plasmin) were normal.

These findings suggest that increased P. F. activity may play a role in the mediating mechanism for the edema in familial hereditary angioedema.

Parameters of Effectiveness of Norepinephrine in Standard Hemorrhagic Shock

By *W. R. Webb, S. S. Lee and J. C. Griffin, Jr.*

Department of Surgery, University of Mississippi School of Medicine, Jackson, Mississippi.

Though clinical experiences have suggested to many observers the salutory effect of vasopressors in oligemic shock, several studies have suggested the use of norepinephrine to be detrimental. The purpose of this study was to re-examine the value and limitations of norepinephrine infusions in standard hemorrhagic shock.

In a preliminary study utilizing nearly 40 dogs, a method was developed to give a standard proportionate reduction of the circulating blood volume with a control mortality rate of 50%. Dogs lightly anesthetized with thiopental were bled into an arterial reservoir set to maintain a pressure of 20 mm. Hg for 20 minutes. The reservoir was then clamped for 90 minutes before reinfusion of the blood. During this period of oligemia, norepinephrine was given to maintain the pressure between 80 and 90 mm. Hg. This required an average of 15 cc./Kg. of norepinephrine in saline (16 μg./cc.). The paired control animals received an identical quantity of saline at the same rate. During the development of methods it was noted that too fast an initial infusion or raising of the blood pressure above 90 mm. Hg almost invariably resulted in cardiac arrhythmias and death immediately. Serial hematocrits, bleeding volumes and dog weights showed no significant differences. Eleven of the 22 control animals died, in comparison to 4 of the 22 experimental animals. Seven controls died during hypovolemia, while only 3 experimental animals died early, apparently from the chronotoxic effect of excess norepinephrine. Eighteen of the 19 norepinephrine animals that survived oligemia were permanent survivors, while 4 control animals died after the conclusion of the experiment.

This study indicates that norepinephrine, when used carefully and in minimal dosages, can be of value in hemorrhagic shock, though higher dosages as used in most previously reported experiments may be deleterious.

Renin and Renin Tachyphylaxis

By *H. G. Langford.* Department of Medicine, University of Mississippi.

The mechanism of renal hypertension remains unknown. Recent studies by Skeggs, Braun-Menenedez et al. have reawakened interest in the renin, renin-substrate, angiotensin mechanism. An embarrassing point for the proponents of the renin mechanism of hypertension is the occurrence of tachyphylaxis; i.e., with continued administration of renin progressively smaller blood pressure responses are obtained, until finally there is no response. Early reports stated that this was due to exhaustion of renin substrate.

We have confirmed the findings of Goldblatt that tachyphylaxis occurs prior to the exhaustion of renin substrate. To study the problem further the approach of Horita and Dille in studying tachyphylaxis to ephedrine was utilized. They found that total peripheral resistance (TPR) remained elevated, while cardiac output (C.O.) and B.P. returned to normal.

In the present experiments, B.P. and C.O. by dye dilution was determined in 7 dogs; then 132 units of renin were infused over 1 hour. C.O. determinations were done at peak B.P. and at 1 hour when B.P. had returned to previous levels.

In all animals the initial response to blood pressure elevation by renin was accompanied by an increased TPR. The final determination, taken when B.P. had returned to control levels despite continued renin infusion, revealed a decrease in TPR in 6 of 7 dogs, though to control levels in only 1. No change occurred in epinephrine responsiveness. This suggested the possibility of (a) a renin antagonist: in previous studies we were unable to demonstrate such a substance. (b) An angiotensin antagonist: this was searched for by determining the pressor response to angiotensin in the rat, then infusing 2 cc. serum from the tachyphylactic dog. The pressor response to angiotensin was markedly reduced or abolished by this maneuver. Control sera caused either no change or slight reduction in responsiveness.

It is suggested that the mechanism of tachyphylaxis to renin differs from that to ephedrine, and may in part be due to the development of an angiotensin antagonist, as suggested by Page and Helmer some years ago. It does not, however, remove the phenomenon as a stumbling block to the renin enthusiasts.

Iproniazid Therapy of Arterial Hypertension

By *Harold H. Orvis and Irene G. Tamagna.* Department of Medicine, George Washington University School of Medicine, Washington, D. C.

That iproniazid (Marsilid) might have an appreciable hypotensive effect on arterial pressure became apparent in early clinical trials with this drug. Although the mechanism of this action

is as yet not clear, preliminary studies have indicated that sustained orthostatic hypotension may be induced in hypertensive subjects. The present study was undertaken to further define the usefulness of this medication, alone and in combination with chlorothiazide, in a small group of patients with well-documented arterial hypertension.

Phases of treatment included: iproniazid, 150 mg. daily, 10 weeks; iproniazid placebo, 8 weeks; chlorothiazide, 1 Gm. daily, 3 weeks; chlorothiazide, 1 Gm. plus iproniazid 75 mg. daily, 8 weeks; chlorothiazide placebo plus iproniazid, 75 mg. daily, 3 weeks.

Sitting and standing blood pressures were recorded at frequent and regular intervals. The following observations were made from the first 7 patients to complete all treatment phases: Six of these 7 patients demonstrated sustained lowering of diastolic pressure averaging 25.5 mm. Hg when on iproniazid, 150 mg. daily, as compared to placebo pressures. Likewise, a decline of diastolic pressure averaging 24.1 mm. Hg was observed when these patients received iproniazid, 75 mg., and chlorothiazide, 1 Gm. daily. Statistical evaluation by analysis of variance indicates these drug effects are not significantly different from each other but result in lower pressures than the placebo at the .005 level of significance.

All patients demonstrated improved mental outlook at the first visit after initiation of iproniazid therapy. Troublesome side effects were not apparent until the 2nd month of therapy when insomnia and nervousness were frequent. Excessive postural hypotension occurred in 2 patients, but reduction of iproniazid dosage enabled continuance of therapy. Side effects with the lower dosage of iproniazid combined with chlorothiazide were mild and required no modification of the dosage schedule. There has been no evidence of hepatic toxicity.

Comment: It was apparent to us that sustained and controllable hypotension can be induced in patients treated with iproniazid alone or in combination with chlorothiazide. Furthermore, we feel that therapy employing iproniazid is simpler than with other potent hypotensive drugs, both with respect to the desired clinical response as well as to troublesome side effects. In view of the potential hepatic toxicity of iproniazid, perhaps the most significant contribution of these initial studies is to suggest a new approach to the mechanism of hypertensive disease.

The Effect of Feeding Unsaturated and Saturated Fatty Acids on Arterial Tissue Cholesterol of the Rabbit

By *Joseph M. Merrill, Bonnie Keith and Walter Earley.* V. A. Hospital, Nashville, Tennessee.

The purpose of this study was to determine the effects of feeding unsaturated (linoleic acid) and saturated (cocoanut oil) fatty acids on serum and tissue cholesterol of rabbits.

Four groups of rabbits were used. Group I was fed a control stock diet. Group II was fed the same diet plus 2% cholesterol. Group III was fed the stock diet plus 2% cholesterol and 10% linoleic acid. Group IV was fed the stock diet plus 2% cholesterol and 10% cocoanut oil. Body weight and serum cholesterol were determined weekly. After 8 weeks of the experimental diets the animals were killed, and tissue obtained from the aorta and liver were analyzed for total cholesterol.

Group I animals had a final average *serum* cholesterol (mg.%) of 52 S.E. ± 8; Group II, 1369 S.E. ± 207; Group III, 2285 S.E. ± 410; Group IV, 1735 S.E. ± 342. Analysis of *arterial tissue* for total cholesterol (mg./100 Gm. fresh tissue) revealed the following: Group 1, 134 S.E. ± 18; Group II, 201 S.E. ± 32; Group III, 435 S.E. ± 106; Group IV, 218 S.E. ± 21. The average *liver* Cholesterol (mg./100 Gm. fresh tissue) was: Group I, 286 S.E. ± 28; Group II, 1754 S.E. ± 405; Group III, 7940 S.E. ± 1380; Group IV, 2260 S.E. ± 506.

The addition of 10% linoleic acid to the diet containing 2% cholesterol caused a 116% increase in the aortic tissue cholesterol. The serum and liver cholesterol increased by 67% and 353%, respectively. Although the cocoanut oil also increased the cholesterol content of the tissues studied, the increment was less than that observed with the linoleic acid. Additional studies have confirmed the striking increase observed in serum cholesterol of rabbits when linoleic acid and cocoanut oil are added to the diet containing cholesterol.

ENDOCRINES AND METABOLISM

Effects of Thyroid Hormone on the Maturation of the Central Nervous System

By *Max Hamburgh*. Departments of Anatomy and Pediatrics, Albert Einstein College of Medicine, Yeshiva University, New York City.

The observation in children with cretinism, that replacement therapy with thyroid hormone may be more effective in raising cerebral function to normal levels if started very shortly after birth, suggests that in the absence of thyroid hormone irreversible changes are taking place in nervous tissue during its period of maturation. The experiments to be reported here were undertaken to answer specifically the following questions:

(1) Are all parts of the central nervous system equally sensitive to the presence or absence of the hormone during its maturation? (2) Is the maturation factor of the hormone required continuously during the period of development of the tissue, or is its action and influence limited only to a very specific and critical phase in the sequence of differentiation?

Mice of the DBA/2 strain were chosen because they are excellent breeders. The experiments were subsequently extended to strains C57 B1/10 and A Jax.

Mice were injected with 2 μg. of triiodothyronine every 2nd day. Injection was started in one series from birth and in another from the 7th day of age. In a 3rd series of experiments, animals were thyroidectomized at birth by injection of 100 μc. of I^{131}.

The age at which the corneal reflex, the startle response, the swimming reflex and the ability to develop a conditioned response emerge were recorded as indicators of completed neural maturation of certain sensory and reflex patterns.

Our results indicate that: (1) Thyroid treatment accelerates, while thyroidectomy delays, the mean age at which the corneal and startle response normally appear. (2) There is no clear evidence of an effect of excess of thyroid hormone or its lack on the emergence of the swimming reflex and the ability to perform a simple conditioned response. (3) Thyroid treatment initiated on the 7th day of postnatal age instead of at birth was equally effective in accelerating those types of behavior that yielded positively to hormone treatment at an earlier age. Conversely, thyroidectomy did not suppress the emergence of any behavior pattern but merely delayed its rate of maturation.

From our results it can be tentatively concluded that the thyroid hormone affects different components of the central nervous system to a different degree, and that some structures may be altogether insensitive to the presence or absence of the hormone.

The further observation that these "acceleration" effects could be elicited if hormone injection was delayed for as long as 7 days after birth suggests to us that during development the hormone might act as a "trigger" activating various components of the central nervous system.

Irreversible Myxedema, With and Without Coma

By *John F. Nickerson, Samuel B. Barker, Jean H. McNeil and S. Richardson Hill, Jr.* Department of Medicine and University Hospital, University of Alabama Medical Center, and Medical Service, V. A. Hospital, Birmingham, Alabama.

Adult myxedema has generally been considered a completely reversible disease if treated. In the natural history of the disease we have observed a stage of hypometabolism in which no available replacement therapy has been satisfactory. In only one previously reported case, however, has parenteral triiodothyronine been used.

In the present study 5 patients are presented in whom a laboratory and clinical diagnosis of myxedema was established and in whom therapy was instituted. They were all females, ages 48 to 71, with a long history of untreated primary hypothyroidism. Two of the patients also had severe concomitant disease (miliary tuberculosis and diabetes mellitus). Because of the inability to take oral medication and the poor intestinal absorption of 3 patients, a parenteral solution of triiodothyronine was given intravenously or intramuscularly in doses of 30 to 100 μg. daily. Dessicated thyroid was given orally in doses of 15 mg. daily to 2 patients. Three patients also received parenteral adrenal cortical glucocorticoids. Four patients showed no response and died, 2 with sudden death, despite vigorous treatment. Autopsies were obtained on 2 who demonstrated small fibrosed thyroids. The one patient who lived was begun on 15 mg. dessicated thyroid per day as she was progressing into a hypothermic coma. Over the next 4 days the patient

showed some improvement of her hypothermia and became somewhat more mentally alert. Intramuscular triiodothyronine (45 μg./day) and cortisone acetate (25 ml./6 hours) were then added to the therapeutic regimen, following which there was gradual recovery.

Myxedema with or without coma is a potentially fatal illness which may reach a stage of irreversibility in spite of the institution of therapy. The present unsatisfactory therapeutic approach should be reevaluated.

The Effect of Parenterally Administered Vitamin B_{12} on Muscular Contraction in Myxedema

By *John D. Lawson and Arthur S. Weissbein.* Department of Medicine, Brooke Army Hospital, Brooke Army Medical Center, Fort Sam Houston, Texas.

The purpose of this study was to determine whether vitamin B_{12} had any effect on the prolonged muscular contraction time observed in myxedema.

The contraction time of the free Achilles reflex was measured with a new electromagnetic device, the Kinemometer, which is so designed as to record essentially movement in only 1 plane, although the movement may be complex with simultaneous components in 3 planes.

Only patients who were clearly myxedematous, or undergoing treatment for myxedema, were studied. The Achilles reflex was recorded before therapy was begun, after the parenteral administration of 1 mg. of B_{12}, and during therapy with U.S.P. dessicated thyroid. During the course of thyroid therapy, 1 mg. of B_{12} was given parenterally prior to any change in dosage of thyroid substance.

In euthyroid and hyperthyroid subjects no change was noted in the Achilles reflex contraction time after the administration of B_{12}. In untreated myxedema the administration of B_{12} caused a lengthening of the contraction time of 40 to 100 milliseconds within 4 hours, which is well outside the limits of experimental error (8 millisec.).

During the course of therapy it was noted that when the dosage was inadequate the administration of B_{12} continued to cause prolongation of the reflex, whereas if the dosage of thyroid was excessive, the administration of B_{12} caused the reflex to *shorten* within 4 hours.

It is concluded that vitamin B_{12} has some unknown effect on the contractile process of skeletal muscles in patients with myxedema, and that when myxedematous patients are treated with dessicated thyroid the response to vitamin B_{12} may be altered in a manner not seen in normal subjects or myxedema patients who have not been treated.

Creatine Metabolism in Hyperthyroidism

By *Coy D. Fitch, Randle Coker and James S. Dinning.* Departments of Medicine and Biochemistry, University of Arkansas Medical Center, Little Rock, Arkansas.

Young Sprague-Dawley rats were made hyperthyroid by feeding diets containing dessicated thyroid or by thyroxine injections. Control and experimental animals were injected with glycine-1-C^{14} or creatine-1-C^{14} and killed after time intervals varying from 30 minutes to 4 hours. The concentrations and specific activities (S. A.) of kidney glycocyamine, and liver, skeletal muscle and heart creatine were determined.

Hyperthyroidism resulted in cardiac hypertrophy and in a greatly reduced heart creatine concentration. The concentration of kidney glycocyamine, liver creatine and skeletal muscle creatine were not significantly influenced by hyperthyroidism. In the glycine-C^{14} injected rats hyperthyroidism resulted in an increased S. A. of liver creatine and a reduced S. A. of skeletal muscle and heart creatine. This indicates a depressed rate of muscle creatine turnover. In the creatine-C^{14} injected animals hyperthyroidism did not appear to affect the S. A. of skeletal muscle creatine, but did result in markedly elevated serum creatine-C^{14} levels. If serum creatine is the immediate precursor of muscle creatine, this also indicates a depressed rate of muscle creatine turnover in hyperthyroidism. It is concluded that there is a block in the incorporation of creatine into skeletal muscle and the heart in hyperthyroid rats.

The Effect of Physical Conditioning on Glucose Tolerance

By *Leo Lutwak and G. Donald Whedon.* Metabolic Diseases Branch, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland.

Increased glucose utilization has been shown to occur during acute exercise, and chronic diseases associated with physical inactivity have been shown to be associated with decreased utili-

zation of orally administered glucose. The subject of the present report is the production of the latter phenomenon and its reversibility by physical conditioning in normal individuals.

Ten normal young adults, 6 females and 4 males, receiving diets containing 200–400 Gm. of carbohydrate a day and at least 2400 calories, were placed at complete bed rest for 1 to 3 weeks. Subsequently, they were placed on a regimen of increasing exercise for 2 to 6 weeks, based on treadmill walking in addition to ad lib. gymnasium activities. Intravenous glucose tolerance tests were performed at rest at intervals prior to and during the bed rest phase and during the activity period. Glucose utilization coefficients were calculated according to the method of Amatuzio et al.

Although a wide inter-individual variability of glucose utilization was observed, in all the subjects complete inactivity produced a marked decrease in glucose utilization within the first week of bed rest with values in the diabetic range. Within a week following institution of the program of physical reconditioning, the utilization returned to base line values, with some individuals showing utilizations far above normal.

Chronic activity, or physical conditioning, results in increased efficiency of the utilization of glucose. Conversely, enforced bed rest leads to impaired glucose utilization.

Oral Diethanolamine Hydrochloride in Short-Term Treatment of Complete Diabetes Mellitus

By *Dan Christian Roehm*. Nashville, Tennessee.

In the accompanying abstract the diverse biochemical effects of the amine-alcohols are cited. From evaluations of the various (aryl-) and alkanolamines, of the 3 ethanolamines, greatest activity is revealed in diethanolamine (DEA) (2,2′ iminodiethanol) suitably neutralized. For one year the impression had been gained that an unrecognized, non-insulin dependent, yet physiologic treatment of diabetes mellitus existed. This has been confirmed. Canines XVII and XIX were alloxanized and depancreatized, respectively. In XIX intravenous DEA·HCL, 0.8 Gm. as the base produced changes in blood sugar in 2-minute intervals as follows: Control 331, 289, 200 and 216 mg.%. In both animals ketonuria disappeared rapidly. In patient N. M., 62 units of insulin were stopped. Replacement with diethanolamine was purposely under-dosed, and at 24 hours blood glucose was 403, ester/total cholesterol 206/330 and uric acid 4.35 mg.%, respectively; the dosage was increased and these became 208, 170/300, 4.10 mg.% in 24 additional hours. Glycosuria was present without ketone bodies. No changes in BUN, K^+ or PO_4 were seen. Oral maintenance is still in progress.

Diethanolamine is glycogenopexic to the liver (I). Its molecular bonding, imine-ol configuration and other biophysical properties may permit peptide linkages, hence enzyme-like effects. Other actions in man are: lipotropism (II), a protein-purine effect possibly centered on hypoxanthine (III). (II) produces increased liver ability to desaturate fats with resultant carbohydrate-sparing, yet (I) is probably a primary phenomenon. (III) may not be overlooked since hypoxanthine is a known lecithinase activator.

Studies on the Use of Chlorpropamide in Patients with Diabetes Mellitus

By *N. Sheldon Skinner, Jr., O. Thomas Bolding, Robert L. Hayes and S. Richardson Hill, Jr.* Department of Medicine and University Hospital, University of Alabama Medical Center, and Medical Service, V. A. Hospital, Birmingham, Alabama.

The toxic and metabolic effects of chlorpropamide given orally in doses of 0.125 to 3.0 Gm. daily for a maximum of 22 weeks were evaluated in 52 unselected patients with diabetes mellitus.

Control of the diabetes was fair to excellent, as judged by blood sugar determinations in 52% of the patients. A general trend developed which suggested that the older the individual at the time of onset of diabetes, the better was the response to chlorpropamide. Chlorpropamide was 8 to 16 times more potent than tolbutamide in controlling diabetes in some patients.

Side reactions included weakness, ataxia, nausea, vomiting, pruritis and skin rash, with an over-all incidence of 37% on doses of 250 to 500 mg. daily; only 15% of these were forced to have the drug withdrawn because of side reactions. No serious hematologic, renal or hepatic toxicity was noted. A direct relationship existed between the size of the dose used and the frequency and severity of the side reactions that occurred.

While on chlorpropamide therapy, 22 patients had a mean decrease in their serum cholesterol level of 41 mg.% (280 to 239). The 9 patients with the highest initial mean cholesterol

level showed a mean decrease of 79 mg.% (324 to 248). The remaining 13 patients showed a mean decrease of 17 mg.% (250 to 233).

The mean decrease in serum protein–bound iodine level in 23 patients following chlorpropamide therapy was 0.4 μg.%. Nine of these patients showed a decrease of over 1.0 μg.%.

The Effect of Endogenous Insulin Secretion upon the Magnitude of Hepatic Binding of Labeled Insulin during a Single Transhepatic Circulation in Human Subjects

By *Norman Kaplan and Leonard L. Madison.* Department of Internal Medicine, University of Texas Southwestern Medical School.

Since endogenous insulin is secreted into the portal vein, traversing the liver before becoming available to extrahepatic tissues, the magnitudes of the hepatic and peripheral effects may depend upon the amount of insulin bound to the liver during its initial transhepatic circulation.

These studies aimed to define the factors controlling hepatic insulin binding by investigating the effects of endogenous insulin secretion upon the magnitude of hepatic binding of labeled insulin. Endogenous insulin secretion was stimulated to varying degrees by varying the duration of an infusion of a 5% glucose solution.

At abdominal operation, a solution containing known amounts of *inulin* and 0.55 units of insulin-I^{131} was injected into the portal vein. Ten seconds later, brachial arterial blood was collected over a 15-second period. Concentrations of *inulin* and tagged insulin were measured and "apparent volumes of distribution" of each determined. From the differences between the "apparent volumes of distribution," hepatic binding of tagged insulin could be calculated.

In 15 human subjects who did not receive glucose prior to intraportal injection, 54% of the injected tagged insulin was bound to the liver in a single transhepatic circulation. As endogenous insulin secretion was progessively stimulated, hepatic binding of insulin progessively declined. In 13 subjects who received 9 Gm. glucose over 17 minutes, hepatic binding fell significantly to 38% of the administered dose. Finally, in 11 subjects who received 52 Gm. of glucose over 167 minutes, hepatic binding dropped to only 7.8% of the amount injected.

The results indicate that when insulin secretion is continuously stimulated by carbohydrate loading, a progressive alteration in the magnitude of the distribution of insulin between hepatic and peripheral tissues occurs. This suggests that when the organism is presented with a carbohydrate load, insulin acts initially by decreasing hepatic output of glucose and later by increasing peripheral glucose utilization.

The Permissive Role of Cortisone in the Inhibition of Hepatic Insulin Binding during Operative Stress

By *Walter Skinner and Leonard L. Madison.* Department of Internal Medicine, University of Texas Southwestern Medical School.

The mechanisms whereby 11-oxysteroids alter carbohydrate metabolism have not been elucidated. Other studies from this laboratory indicate that abdominal laparotomy enhances the effect of exogenous insulin on peripheral glucose utilization. The role of operative stress and 11-oxysteroids in the distribution of insulin between hepatic and peripheral tissues was investigated by determining their effect on hepatic binding of insulin during a single transhepatic circulation in 47 rats.

Four groups were studied: Group I—12 control rats; Group II—10 adrenalectomized rats maintained on a fixed dose, (0.3 mg./Kg./day) of cortisone; Group III—8 adrenalectomized rats given 0.5 mg. DOCA/day and 2 mg./Kg. of hydrocortisone immediately prior to the experiments; Group IV—17 adrenalectomized rats maintained on DOCA alone.

Ten seconds after the administration of 0.052 units of I^{131}-labeled insulin into the portal vein the rats were sacrificed. Immediately thereafter the livers were perfused with 50 ml. of Ringer's lactate, and bound hepatic radioactivity, expressed as % of administered dose, was measured in a deep-well counter. Abdominal laparotomy, prerequisite for endoportal insulin administration, constituted the operative stress.

The results indicate that adrenalectomized rats given DOCA only (Group IV) bound 45% of the administered insulin in their livers during a single transhepatic circulation. By contrast, each group having access to either endogenous corticosteroids (Group I) or exogenous corticosteroids (Group II and III) bound significantly less of the administered insulin, i.e., 28%, 29.3% and 29.4%, respectively.

Since this 33% decrease (from 45 to 29%) in hepatic insulin binding during operative stress

required only small fixed doses of cortisone, the 11-oxysteroids may be considered to have only a "permissive effect" in bringing about this change. Shunting of insulin from liver to periphery may explain the enhancement of the effect of exogenous insulin upon peripheral glucose utilization during operative stress.

The Effect of Denervation on the Glucose Uptake and Insulin Sensitivity of the Isolated Rat Diaphragm

By *John Buse and Maria Gordon Buse.* Department of Medicine, Medical College of South Carolina, Charleston, South Carolina.

Exercise is known to decrease the insulin requirements of diabetics and to facilitate the penetration of certain monosaccharides into muscle cells. The mechanism of action is not clear.

The in vitro glucose uptake and insulin responsiveness of intact and paralyzed hemidiaphragms 12 to 200 hours after unilateral denervation were compared in this study. Hemidiaphragms were incubated in buffer (Gey and Gey) containing 300 mg. % glucose. The amount of glucose consumed during 90 minutes, incubation was related to the tissues, dry weight.

The glucose uptake (glucose mg./100 cc./10 mg. tissue dry weight) of paralyzed (P) and control (C) hemidiaphragms was identical in the absence of insulin (P = 21.7 ± 1.5; C = 21.3 ± 1.1). In all cases, ± refers to S.E.M. When 0.001 units/cc. were added to the medium, paralyzed hemidiaphragms consumed less glucose than controls (P 12 hours = 29.4 ± 0.7; P 40–200 hours = 20.5 ± 1.2; C = 35.1 ± 1.5). Dose response studies indicated that the insulin sensitivity decreased to 1/10 of normal 12 hours after denervation. Sham-operated rats were identical with controls.

Unilaterally phrenicotomized rats received 0.1 units of insulin i.v. After 30 minutes the hemidiaphragms were excised and incubated. Paralyzed hemidiaphragms consumed less glucose (28.9 ± 3.1) than controls (38.6 ± 2.3).

The insulin-binding capacity of paralyzed and control hemidiaphragms was compared (a) during incubation with I^{131} insulin and (b) after i.v. I^{131} insulin. No difference in insulin binding was observed.

Determinations of muscle glycogen showed that paralyzed hemidiaphragms of fasted rats contain more glycogen (glycogen as glucose mg./Gm. wet weight) (1.99 ± 0.12) than their working pair (1.18 ± 0.09). After feeding, the glycogen content increased 30% in paralyzed hemidiaphragms and 200% in controls (P = 2.63 ± 0.20; C = 3.73 ± 0.17).

The insulin-binding capacity of paralyzed hemidiaphragms is unimpaired, while their metabolic response to the hormone is decreased, due to inactivity or denervation. Decreased insulin sensitivity preceded detectable morphologic changes in muscle.

Adrenal Blood Studies in Man: Epinephrine, Norepinephrine and 17-21 Hydroxycorticoid Levels in Peripheral and Adrenal Vein Plasma

By *Thelma E. Carter and James D. Hardy.* Department of Surgery, University of Mississippi Medical Center, Jackson.

The purpose of the present study was to measure the concentrations of epinephrine and norepinephrine in human adrenal vein plasma. In addition, the adrenal vein catechol amine levels were compared with those of peripheral blood, and the changes in plasma catechol amine levels were compared with those for plasma 17–21 hydroxycorticosteroids.

Serial epinephrine (E) and norepinephrine (NE) levels in peripheral plasma were determined (method of Weil-Malherbe and Bone) in 13 patients and in adrenal vein blood taken during laparotomy in 7 patients. Serial plasma 17–21 hydroxycorticoid values in peripheral blood were measured in 9 patients and in adrenal vein blood in 7 patients, in addition to more than 20 patients so studied and previously reported.

The average peripheral plasma catechol amine levels preoperatively were E 1.0 and NE 4.2 μg./L.; during operation, E 1.6 and NE 3.6; 4 hours following operation, E 1.8 and NE 4.2; one day after operation, E 1.4 and NE 4.3. The average concentration of epinephrine in adrenal vein blood at surgery was 14.2 μg./L., whereas that of norepinephrine remained approximately the same as the peripheral concentration. The average adrenal vein output of epinephrine was estimated to be 0.226 mg./24 hrs.

Conclusions: Operation consistently increases the plasma level of epinephrine, but not of norepinephrine. The norepinephrine concentration in adrenal vein blood is approximately the same as that in peripheral blood.

Adrenocortical Function after Long-Term Corticoid Therapy

By *Gabriel G. Carreon, John J. Canary and Laurence H. Kyle.* Departments of Medicine and

Biochemistry, Georgetown University School of Medicine and Georgetown University Hospital.

This study was designed to test the postulated parallel between the prolonged suppression of adrenocortical function noted after removal of unilateral cortical tumors and that following cessation of long-term glucocorticoid therapy.

Study was made of 3 patients who had received 37.5 to 75 mg. of glucocorticoid daily for from 2½ to 7 years. Corticoids were abruptly withdrawn and daily 24-hour urine levels of neutral 17-ketosteroids and 17-hydroxysteroids measured. After 3 to 6 days without exogenous corticoids, the adrenal response to a standard 8-hour intravenous ACTH infusion was tested. Sustained stimulation was then effected by the administration of intramuscular ACTH Gel every 12 hours for from 4 to 5 days. An 8-hour stimulation test immediately followed and was again performed 4 to 11 days later.

Throughout the peroid following abrupt corticoid withdrawal, the urinary 17-OHCS and 17-KS levels in each subject were unequivocally low. The response to an 8-hour intravenous stimulation at the end of this period was subnormal in all. Sustained ACTH stimulation induced elevated excretion levels of corticosteroids. A repeat 8-hour stimulation then evoked a normal response in all subjects. Following this there was immediate regression of 17-OHCS and 17-KS excretion to pre-stimulation levels, and 2 of the 3 subjects demonstrated an inadequate response to the 8-hour ACTH test.

Unequivocally hypoadrenal levels of steroid excretion after corticoid withdrawal and subnormal response to a single ACTH stimulation indicate marked adrenocortical suppression. Restoration of adrenal activity with sustained stimulation implies integrity of the adrenal cortex. Immediate regression to and persistence of a hypoadrenal state after discontinuation of exogenous stimulation connotes deficiency of endogenous ACTH production in these patients. Administration of ACTH following prolonged corticoid therapy offers no assurance of permanent restoration of adrenocortical function.

The Renal Clearance of Endogenous Free and Conjugated 17-Hydroxycorticosteroids in Normal and Hypertensive Subjects

By *Ludwig Kornel*. Department of Medicine and University Hospital, University of Alabama Medical Center, and Medical Service, V. A. Hospital, Birmingham, Alabama.

Renal clearance of endogenous free and conjugated 17-hydroxycorticosteroids (17-OH-CS) has been determined in 15 normotensive and 13 hypertensive subjects (12 with essential hypertension and one with Cushing's syndrome). The free 17-OH-CS are cleared at similar rates in the 2 groups of subjects, 2.0–9.0 ml. of plasma per minute (mean 4.9 ml.), which constitutes 2.0–10.0% of the creatinine clearance determined simultaneously. The total conjugated 17-OH-CS (extractable with butanol-chloroform mixture) are cleared in normotensives at rates of 90–189 ml. of plasma per minute (66–140% of creatinine clearance), mean 127 ml. (98% of creatinine clearance); in hypertensives at rates of 31–90 ml. of plasma per minute (32–79% of creatinine clearance), mean 57 ml. (51% of creatinine clearance). The 17-OH-CS glucuronides (released by beta-glucuronidase hydrolysis) are cleared at similar rates in normotensive and hypertensive subjects; in individuals with decreased renal function these rates are proportional to those of the creatinine clearance. Moreover, the concentrations of 17-OH-CS glucuronides in urines of hypertensive patients are almost identical with the concentrations of total conjugated 17-OH-CS in the same specimens.

The decreased clearance of the total conjugated 17-OH-CS leads to their accumulation in plasma with a resulting alteration in the ratio of free to conjugated 17-OH-CS in plasma. The mean of this ratio was 1.1 in normotensives and 2.2 in hypertensives, with P less than 0.001.

On ACTH, the clearance of free 17-OH-CS markedly increases in both groups of individuals. The clearance of total conjugated 17-OH-CS on ACTH decreases in normotensives but slightly increases in hypertensives, as compared with the corresponding clearances under basal conditions. Thus, the hypertensives under basal conditions clear the total conjugated 17-OH-CS in a manner similar to the normotensives on ACTH.

A possibility of an altered pattern of conjugation as a factor responsible for the mechanism of the detected differences is suggested.

Electrical Anesthesia: Observations of Adrenal Activity and Comparison

By *C. D. McNeil, M. D. Turner and J. D. Hardy*. Departments of Surgery and Biochemistry, University of Mississippi Medical Center, Jackson, Mississippi.

Adrenal medullary and adrenal cortical response to electrical anesthesia was compared with

2 familiar general anesthetic agents, ether and pentobarbital.

Twenty-eight dogs were divided into 4 groups of 7 animals. Three groups were subjected to different general anesthetic agents and a standard celiotomy of 30-minute duration was performed. Anesthesia was induced in one group by an electrical current passed through the head between 2 electrodes, in another group with pentobarbital and in a 3rd with ether. The remaining group received only procaine locally. The latter was considered a control group. Blood samples were obtained before, mid-way and at the end of the operative procedure and 1, 2 and 2½ hours after. All plasma samples were analyzed for 17,21-dihydroxycorticoids, epinephrine and norepinephrine.

Electrical anesthesia produced an increased elaboration of the above substances considerably above that observed in the other groups. Ether anesthesia had little effect on the secretion of the measured substances. Pentobarbital produced a depression of adrenal cortical and adrenal medullary activity in animals subjected to surgical stress. Alterations in norepinephrine levels were found to be delayed in time.

Conclusions: Electrical anesthesia subjects an animal to severe stress and appears to be more traumatic than the stress of surgery. This agent does, however, permit the adrenal medullary and adrenal cortical systems to respond freely. Pentobarbital appears to have a depressive effect on the systems studied. It is too early to suggest clinical applicability.

Uses of the N-Alkanolamines in Treatment of Diseases of Metabolism: Gallogen, Deaner, Diethanolamine (DEA) and Glucosamine HCl

By *Dan C. Roehm and J. Jeff Hooberry, Jr.*
Nashville, Tennessee.

Three-hour diagnostic oral glucose tolerance tests (A) of 6 consecutive, newly diagnosed, adult-onset diabetics were repeated (B) after 3 to 140 days of Gallogen perle therapy. One 250 mg. Gallogen perle (DEA salt) was swallowed 1 hour before (B); all other factors were carefully controlled. Each patient's tolerance was found more nearly normal. Three-hour mean blood sugars (Folin-Wu) were after 100 Gm. oral glucose (A) and (B), respectively: 113.0 ± 13.2—114.2 ± 13.3; 189.9 ± 31.—171.8 ± 27.9; 211.7 ± 40.—164.3 ± 40.1; 166 ± 38.9—135.8 ± 26.8; 127.7 ± 36.3—116.2 ± 39.1 mg%. The area subtended by (A) is twice area (B). Glycosuria was present in 1 specimen only during (B) and in 5 patients at (A).

Despite insulin, 150 units daily, a juvenile diabetic developed ketoacidosis and received intravenously 3.4 Gm. DEA. In 2 hours blood glucose 328 mg.% declined to 178 mg.%, and glycosuria and ketonuria disappeared. Next, pure diethanolamine orally for one week reduced the insulin requirement from 150 to 35 units. In 14 days tolbutamide, 2 Gm. daily, failed to reduce the 150-unit requirement.

A gouty patient received 1.4 Gm. DEA intravenously. In 75 minutes blood uric acid fell from 4.6 to 4.1 mg.%, not explained by a moderate uricosuria (0.08–0.10 mg./min.). No acute podagra was precipitated. Remission on Gallogen perles followed. Intravenous Deaner 180 mg. lowered blood urate from 4.75 to 3.88 mg.% (Benedict modification of Brown's method) in only 40 minutes.

A 4-month response in atherosclerosis to Gallogen was A.I. (Gofman): 190, 170, 140, 110, 105 and 95 units, while total serum lipids (Mata): 1,950 mg.% fell to 1,080 mg.%. Liver sections indicate lipotropism in another patient.

In canines glucosamine HCl doubled serum lecithin in 1 hour. Diethanolamine and monohydroxyethylisopropylethylenediamine are also potent lecithin builders (L/P = 26) and can produce fatal hypoglycemia. Preparations must be NH_3-NH_4^+—free.

Alterations in Serum Cholesterol and Endocrine Function Induced by a New Phenothiazine Derivative

By *Mervin L. Clark and Phillip C. Johnson.* Medical Service, Central State Hospital, Norman, Oklahoma, and University of Oklahoma Medical Center and V. A. Hospital, Oklahoma City.

During a clinical trial of a new phenothiazine derivative as a tranquilizer, it was noted that all 3 female subjects stopped menstruating. This effect was not accompanied by an increase in urinary FSH excretion, but an elevation of serum cholesterol did occur. To investigate this phenomenon further, a new experiment was designed in which the drug was administered to an additional 4 female subjects whose menstrual cycles were known to be regular. Control studies of various endocrine functions were made, and observations were continued at appropriate intervals throughout the period of drug adminis-

tration and for 4 months thereafter. The medication period extended over 12 weeks in doses as high as 1.0 Gm./day.

Amenorrhea lasting 4 to 5 months occurred in 3 of the subjects, the normal cycle returning 2 to 3 months after discontinuing the agent. The 4th subject developed metrorrhagia during the drug period. These menstrual disturbances were accompanied by normal to low estrogenic activity reflected in vaginal smears and normal to low urinary FSH excretion. Twenty-four hour I^{131} uptake was depressed slightly (mean, −3%) during the drug period and returned to base line level after the drug was stopped. The serum cholesterol rose in all subjects 31, 60, 93 and 126 mg.%, respectively. Twelve weeks after the agent was stopped the values had fallen substantially in all cases. The PBI remained in the low normal range during the treatment period. There was no clinical evidence of hypothyroidism or adrenal insufficiency.

The data suggest that the drug produced amenorrhea through a mechanism other than direct ovarian depression. It is unlikely that the substantial rise in serum cholesterol can be accounted for by the slight depression in thyroid function. An effect on the pituitary, either direct or indirect, is the most attractive explanation at this time.

In Vitro Synthesis of Lipids from C-14 Acetate by Adipose Tissue from 4 Types of Obese Mice

By *Guy Hollifield, William Parson and Carlos R. Ayers.* Department of Internal Medicine, University of Virginia School of Medicine, Charlottesville, Virginia.

The hyperphagia and obesity following injury to the ventro-median nuclei of the hypothalamus by electro-coagulation or injection of gold thioglucose in mice is the result of interference with the centrally mediated satiety response. Mayer has called this "regulatory" obesity in contrast to "metabolic" obesity which results from "inborn or acquired errors of metabolism of tissue other than the regulating centers."

Studies from this laboratory indicate that 2 strains of mice with hereditary obesity (congenitally obese [ob ob]) and yellow (A^y) have intact hypothalamic feeding centers. Mayer et al. have shown that congenitally obese mice incorporate more injected C-14 labeled acetate in liver and carcass fatty acids than controls in a wide variety of nutritional states. Fasted gold thioglucose obese mice and mice with hypothalamic lesions did not show increased incorporation of acetate.

We have studied the in vitro synthesis of lipids from C-14 labeled acetate by adipose tissue from 4 types of obese mice using the technic described by Baruch and Chaikoff. Mice with congenital obesity (ob ob), yellow mice (A^y), gold thioglucose mice and mice made obese by the subcutaneous implantation of pellets of 11-dehydrocorticosterone with appropriate controls were studied after fasts of 14 to 16 hours. Adipose tissue from congenitally obese (ob ob) mice incorporated over 4 times as much C-14 labeled acetate per mg. of nitrogen and 11-dehydrocorticosterone treated mice over 2 times as much as adipose tissue from their controls ($P > .01$). Adipose tissue from gold thioglucose obese mice and yellow mice did not show significantly greater incorporation of acetate than that from controls.

The increased in vitro lipid synthesis by adipose tissue of congenitally obese and 11-dehydrocorticosterone treated mice supports the concept of "metabolic" obesity. This increase in lipid synthesis is not due to obesity since equally obese yellow and gold thioglucose mice do not show it.

Physiologic Derangements in Patients with Hyperkalemic Familial Periodic Paralysis

By *R. Klein and T. J. Egan.* Department of Pediatrics, University of Pittsburgh Medical School.

We have studied the alterations in electrolyte, water and sugar metabolism in hyperkalemic familial periodic paralysis (FPP) in order to suggest an explanation of its pathogenesis.

Measurements were made of AV differences of sodium, potassium, phosphorus and sugar in the forearm. Other plasma constituents were measured only in venous blood. Venous red cell sodium and potassium and water content were calculated from hematocrit and whole blood and plasma determinations.

Attacks of paralysis in these patients are ushered in by rising red cell water, falling plasma water and rising plasma potassium, often with a negative AV difference in the forearm. The red cell potassium falls as the cell sodium rises. Recovery reverses these changes.

Glucose, given to normal individuals, lowers arterial serum potassium and increases peripheral uptake. Grob has shown an exaggerated re-

sponse in patients with hypokalemic FPP. Glucose ingestion by patients with hyperkalemic FPP produced a greater than normal fall in arterial serum potassium, but no increase in peripheral uptake. At times there is actual release of potassium from the forearm. In normals, epinephrine usually produces a lower arterial potassium and greater peripheral uptake, albeit less than that seen in hypokalemic FPP. In our patients there is a marked and dramatic fall in arterial potassium, but little or no increase in peripheral uptake. Glucagon was twice given to one of our patients. On one occasion an attack had just started. There was a dramatic fall in arterial potassium and an exaggerated increase in peripheral uptake. After recovery from another attack, glucagon was followed again by a fall in arterial potassium, but venous potassium rose to a level above the arterial.

Three of these patients had an abnormally large amount of urinary material that caused sodium diuresis when injected into rats. The children have an increased urinary adrenal corticoid excretion.

Our current hypothesis is that these patients have an excess of a material, perhaps an adrenal hormone, that inhibits the transport of sodium out of cells. This is associated with a decreased cellular potassium, and when serum potassium is raised by ingestion, glycogenolysis or other factors, the increased ratio of extracellular to intracellular potassium causes paralysis. Dexedrine effectively reverses this process and clinically prevents attacks.

GASTROINTESTINAL SYSTEM

Osmotic Pressure of Saliva

By *Samuel J. Friedberg and Eleanor M. Doyle.*
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The following study was undertaken to demonstrate osmotic changes in saliva following dehydration and the i.v. administration of hypertonic saline.

Fourteen determinations were performed on 10 normal male subjects. The saliva was ejected into graduated centrifuge tubes every 30 seconds for 10 minutes. Osmotic pressures and volumes were determined on samples collected before and after dehydration produced by sweating for varying intervals, usually 4 hours, under a heat lamp or in a hot box. At hourly intervals, samples were collected and body weight recorded. The volume produced in each 10-minute collection was recorded and the osmotic pressure determined with the Fiske Osmometer.

In all cases there was a rise in osmotic pressure of saliva. In most, the rise was progressive and varied from 7.5 mOsm./L. to 125 mOsm./L. and was related in a rough way inversely to the reduction in volume of secretion and directly to the weight loss. The average initial salivary osmolality was 69 mOsm./L. and the average rise 39 mOsm./L. ($P < .01$).

In order to control the experiment and eliminate heat as a factor, the procedure was repeated in 6 cases under the same conditions, except that a water load was administered hourly during the period of dehydration to replace weight loss. Under these circumstances there was a drop in salivary osmolality in 5 subjects and a small rise in 1 subject. The average value changed from 71 mOsm./L. to 64.5 mOsm./L. Volume remained about the same under these circumstances. In comparing the final results with and without water, P was less than .01.

In another experiment 6 subjects were rapidly given 350 to 400 ml. of 5% sodium chloride i.v. In all cases, thirst occurred with concomitant rise in saliva osmolality, averaging 37 mOsm./L. and reduction in saliva volume ($P < .01$).

The conclusion reached is that thirst produced by dehydration and the administration of hypertonic saline is accompanied by a rise in the osmotic pressure of saliva.

The Role of the Stomach on Fat Digestion and Absorption

By *W. G. Gobbel, Jr., and H. H. Shoulders, Jr.*
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The role of the stomach in fat digestion and absorption was studied in dogs and in man by administering a fat emulsion of peanut oil and triolein I^{131} and determining the amount appearing in the blood at hourly intervals for 6 hours thereafter. In man the total amount excreted in 48 hours in the stool was also measured.

The test fat meal was given to 2 groups of

dogs: (1) normal (controls), and (2) post-total gastrectomy with esophagoduodenal reconstruction. The degree of digestion and absorption as measured by the total blood radioactivity was approximately the same in the 2 groups, suggesting that the stomach plays no essential role in fat digestion and absorption.

In man the test meal was administered (1) orally, (2) via Cantor tube directly into the duodenum, and (3) via Cantor tube directly into the jejunum for a total of 32 patient determinations. The degree of digestion and absorption was the same following oral and duodenal administration, but following direct jejunal feedings the levels were only ½ as great. The fecal loss was 3.8% of the total dose following oral, 1.6% following duodenal, but 34.8% following jejunal intake.

The observations in man and dogs that fat administered directly into the duodenum appears in the blood to the same degree as that administered directly into the stomach, and that in man the fecal loss is equally small, suggests that the stomach is not necessary per se in fat digestion and absorption.

Effect of Pre-feeding of Fat on I^{131} Triolein Absorption in Intubated Normals and Subtotal Gastrectomy Patients

By *Benno Janssen, Jr., Malcolm P. Tyor, Edward E. Owen and Julian M. Ruffin.* Department of Medicine, Duke University Medical Center, and V. A. Hospital, Durham, North Carolina.

The oral administration of a lipid emulsion containing I^{131}-labeled triolein to patients with subtotal gastrectomy (Billroth II) frequently yields abnormally high concentrations of fecal radioactivity (>4.0% of dose/48 to 72-hr. collection period). Following intraduodenal injection (3.5 ml./min.) of I^{131} triolein, elevated fecal radioactivity has been found in normal subjects. The purpose of the present study was to: (1) extend these observations in intubated normals, (2) attempt to correct this induced defect, and (3) apply these principles to patients with subtotal gastrectomy.

Eighteen normal subjects received the I^{131}-tagged lipid emulsion intraduodenally; 17 had concentrations of fecal radioactivity >4.0% of dose (group mean = 20.5 ± 12.1%). Eight normal subjects were studied in a similar manner, except that the intraduodenal injection was preceded 30 minutes by the oral ingestion of 50 ml. of a nonradioactive lipid emulsion; 7 subjects had concentrations of fecal radioactivity <4.0% of dose (group mean = 2.1 ± 2.0%). The tagged triglyceride was administered orally to 12 patients with subtotal gastrectomy (Billroth II) on 2 occasions; (1) in the fasting state, and (2) 30 minutes after the oral ingestion of untagged lipid. When lipid was pre-fed, there was a significant decrease in the radioactive content of stool (mean difference = 18.3% of dose, $p = <0.01$) and increase in blood radioactivity ($p = <0.01$).

The data suggest a similar mechanism for the induced defect in intubated normals and patients with subtotal gastrectomy who demonstrate increased fecal radioactivity following I^{131} triolein. In addition, they support the concept that the defect exhibited by patients with subtotal gastrectomy is primarily digestive and suggest that the major disturbance is one of inadequate mixing of the labeled triglyceride with the upper intestinal contents necessary for proper lipid digestion.

An in Vitro Test for a Hydrochloric Acid Inhibitor in Human Gastric Content

By *B. Hill Britton, Jr. and Stewart Wolf.* Department of Medicine, University of Oklahoma School of Medicine, Oklahoma City.

Three groups of independent investigators (Brunschwig et al., Code et al. and Smith et al.) have demonstrated that the intravenous injection into Heidenhain pouch dogs of a preparation of normal human gastric content inhibits powerfully the secretion of hydrochloric acid. This property was found to be shared by saliva but was not present in other mucinous substances such as hog vitreous humor, ovarian cyst fluid and joint fluid. The present study concerns itself with an attempt to develop an in vitro test for the inhibitor material in gastric juice and saliva. In 38 separate experiments the isolated gastric mucosa of a frog was suspended in a chamber between 2 nutrient solutions after the manner of Davenport. After suitable stimulation by histamine and carbachol such preparations continued to secrete hydrochloric acid from the mucosal surface for 5 to 6 hours. Determinations of pH were made on the solution on the mucosal side each hour, and the millimols of hydrochloric acid secreted were calculated. Control runs were compared with those in which 2 mg. of a preparation of human gastric juice were added to the nutrient fluids after the 2nd hour of secretion. In each of 14 such tests

and in 4 experiments using a preparation of saliva there was evidence of substantial inhibition usually amounting to almost complete cessation of hydrochloric acid secretion after the test preparation was added. In further control experiments no such inhibitory effect was noted when another mucinous substance, hog vitreous humor, was used. It appears, therefore, that the frog method is adequate as an in vitro test for the demonstration of a substance in normal gastric content and saliva which powerfully inhibits hydrochloric acid secretion by the gastric mucosa.

The Effect of Pancreatectomy on the Incidence of Gastric Ulceration in the Shay Rat

By *Rene B. Menguy and Merlin K. Duval, Jr.* University of Oklahoma Medical Center, and V. A. Hospital, Oklahoma City.

The relationship of the pancreas to peptic ulcer remains obscure. The importance of this relationship has received new emphasis with the recent description of a severe ulcer diathesis in association with certain pancreatic tumors. The purpose of this study was to determine whether or not pancreatectomy would provide protection against peptic ulceration in the Shay rat preparation.

Ligation of the pylorus of a fasting rat results in rapid development of multiple gastric ulcers (Shay rat). More than 150 of these preparations, with and without simultaneous random removal of the pancreas, have been carried out. Gastric analyses, blood sugar levels and ulcer counts were determined at 24 hours. None of the depancreatized animals became diabetic during the period of observation.

Our data showed that simultaneous pancreatectomy provided almost complete protection against gastric ulceration in the Shay rat preparation. Control experiments in which the flow of pancreatic juice to the duodenum was interrupted, the pancreas being left *in situ*, showed that the protective mechanism of pancreatectomy was not due to the removal of pancreatic juice from the duodenum.

The data lent some support to the concept of an internal factor of pancreatic origin stimulating the production of HCl by the stomach.

Uropepsin Excretion in the Differential Diagnosis of Gastrointestinal Hemorrhage

By *Alvin J. Cummins.* Gastrointestinal Laboratory, Department of Medicine, University of Tennessee, and John Gaston Hospital, Memphis.

There is need for a simple reliable test to aid in the rapid differential diagnosis of the cause of gastrointestinal hemorrhage. If peptic ulcer can be identified early, medical and/or surgical therapy can be applied more rationally. Uropepsin excretion in patients with duodenal ulcer has been reported to be increased, probably reflecting gastric hypersecretion. The present study was designed to determine the feasibility of using a rapid 2-hour uropepsin excretion determination in distinguishing ulcer from non-ulcer hemorrhage.

Voided or catheterized 2-hour urine specimens were obtained, and uropepsin determined by the Anson-Mirsky method. In ⅔ of the cases urinary creatinine was also determined and uropepsin/creatinine ratios calculated to serve as a check on urine collection errors. One hundred and twenty-four patients with gross hemorrhage from the gastrointestinal tract were studied. Of these, 68 had peptic ulcer (11 gastric, 57 duodenal), 32 had hemorrhage not of ulcer origin (4 varices, 8 gastric carcinoma, 5 hiatus hernia, etc.) and in 24 the cause of bleeding was undetermined. The range of 2-hour uropepsin excretions in these patients was as follows: ulcers, 34–1925 units (mean = 638); non-ulcers, 0–1337 units (mean = 272); undiagnosed, 24–1650 units (mean = 503). Similar scatter was observed for the uropepsin/creatinine ratios.

It is apparent that while the ulcer patients as a group have an increased uropepsin excretion the wide range of excretion limits the usefulness of the test in differential diagnosis in the individual patient. A 2-hour excretion of over 600 units, however, strongly suggests the diagnosis of ulcer, since only 2 of the 32 non-ulcer patients showed such values. In no cases except those with duodenal ulcer was the the 2-hour excretion over 1400 units. The test applied would appear to be a valuable but limited tool in the diagnosis of hemorrhage due to ulcer.

Motility and Mortality Following High Cervical Vagotomy Combined with Gastroenterostomy

By *W. R. Webb, R. D. Sloan and S. S. Lee.* Departments of Surgery and Radiology, University of Mississippi School of Medicine, Jackson, Mississippi.

Long-term studies of the physiologic effects of bilateral vagotomy in animals have been hampered by the poorly understood tendency for dogs to die within 2 or 3 days following bilateral cervical vagotomy. Our previous studies sug-

gested that the mechanism of death was primarily an aspiration pneumonitis from regurgitation due to esophago-gastric disfunction.

Vagotomies were performed in 14 dogs, all of which died within 4 days following surgery, with retching, regurgitation and pathologically proven pneumonitis. Pre- and postoperative barium studies were performed to demonstrate the changes of esophageal motility that occur following vagotomy. Postoperative barium studies revealed a virtual paralysis of the lower esophagus. The infrequent peristaltic wave that did occur, however, would carry the barium through the cardia, which demonstrated a normal receptive relaxation.

Ten dogs were subjected to gastroenterostomy, and some weeks later subjected to a bilateral cervical vagotomy. Nine of the 10 dogs were permanent survivals. These dogs, for at least 3 months following vagotomy, showed definite retardation of esophageal motility, but again a normal esophago-cardiac sphincteric relaxation.

From these studies death following high cervical vagotomy in the dog would appear to be due to an aspiration pneumonitis, secondary to regurgitation of esophageal and gastric contents. This can be prevented by relief of the gastric obstruction that ensues following vagotomy, since gastric drainage reduces the quantity and acidity of the vomitus which might be aspirated.

Gall Bladder Mucoproteins in Cholelithiasis

By *Robert B. Giles, Jr.* Department of Internal Medicine, University of Texas Southwestern Medical School, Dallas.

These studies were undertaken to determine whether the mucoproteins secreted by the gall bladder are correlated with cholelithiasis. Gall bladder contents obtained at post mortem and at operation were analyzed for total hexosamines by the Elson-Morgan reaction, total hexoses by the Anthrone method, protein and nonprotein nitrogens by the Kheldahl method after sodium tungstate precipitation. Hexosamines were identified by ion exchange chromatography, and hexoses by paper chromatography. These data were correlated with cholelithiasis and the histologic appearance of the gall bladder mucous membrane.

Hexosamine concentrations in the stone group averaged 57 mg. % as compared to 150 mg. % for the normal group. No significant amount of hexosamine was detected in several stones, either pigment or cholesterol in type. Hexosamine chromatography revealed glucosamine and galactosamine in a ratio of 22 to 1, which did not vary significantly among several normal and abnormal specimens. Total hexose concentrations averaged 174 mg. % in the stone group as compared to the normal of 455 mg. %. Chromatography revealed galactose and fucose in a ratio of 4 to 1. No hexose was found in several stones. The ratio of hexosamine to hexose averaged 0.33 without significant difference between the 2 groups. Total nitrogen and sodium tungstate precipitable nitrogen concentrations of the 2 groups differed in a similar fashion, being much lower in the stone group than in the normal group.

These studies establish that there is a significant difference between the mucoprotein concentration in normal postmortem gall bladder bile and in that associated with cholelithiasis, whether obtained at post mortem or operation. No significant amount was found in gall stones, and the low concentrations found in biles associated with cholelithiasis may reflect disease of the mucous membrane. Whether low mucoprotein concentrations contribute to, or result from, cholelithiasis has not been established.

Chemical and Enzymatic Studies of Normal and Diseased Human Liver

By *John T. Sessions, Jr., W. Geoffrey Wysor, Jr., Nathan A. Womack and Oscar L. Sapp, III.* Departments of Medicine and Surgery, University of North Carolina School of Medicine, Chapel Hill, North Carolina.

Normal and diseased human liver was studied by cytochemical methods to establish normal values for selected elements of the liver cells, to assay activity of enzyme systems and detect changes that occurred with disease.

Liver was secured during celiotomy, the duration and type of anesthesia being noted. Tissue was studied promptly or quickly frozen. Following homogenization, spectrophotometric methods were used in measuring the compounds and enzymes studied.

Average values found in 4 patients without clinical or histologic evidence of liver disease follow: .473 mg. of DNA phosphorus/Gm. of liver; .840 mg. of RNA phosphorus/mg. of DNA-P; lactic dehydrogenase 182.1 x 10^{-3} $\triangle$ μM DPNH/min./mg. DNA-P; succinic dehydrogenase 22.2 x 10^{-3} $\triangle$ μM $K_3Fe(CN)_6$/min./mg. DNA-P; 30.1 mg. N/mg. DNA-P.

Patients with liver disease studied to date include 6 with cancer, 3 with cirrhosis and 1 with hepatitis. DNA values regularly fell in the

normal range. RNA and total nitrogen levels varied widely without relation to serum protein values. Lactic dehydrogenase values were almost all depressed but, in contrast, succinic dehydrogenase activity was elevated, once in cancer, once in hepatitis and twice in cirrhosis.

DNA content was found advantageous as an index of cellularity in normal and diseased liver. Wide variations in cell nitrogen content found in liver disease were associated with clear-cut changes in activity of the enzymes studied. The relative importance of enzyme-substrate relationships, as well as co-factors, in causing changes in enzyme activity could not be determined. However, the clear-cut changes found in disease encourage further use of cytochemical methods in extending understanding of liver metabolism.

Alterations in Liver and Serum Enzyme Activities under Conditions of Portal Venous Ischemia, Hepatic Venous Congestion and Common Bile Duct Ligation

By *Arthur Ruskin, Marcel Patterson, John Sinclair, Fred Wolma, Lee Haasis and Belle Ruskin.* Departments of Internal Medicine, Anatomy and Surgery, and Tissue Metabolism Research Laboratory, University of Texas Medical Branch, Galveston, Texas.

Previously we demonstrated a marked parabolic rise in serum glutamic oxalacetic transaminase (GOT) and lactic dehydrogenase (LD) activities following liver ischemia from near-complete deprivation of arterial blood supply to the liver. This was generally accompanied by a drop in the activities of the same enzymes in the necrotic liver.

To demonstrate the contrasting effects of hepatic venous congestion, deprivation of portal blood supply and bile stasis in the liver, a total of 29 dogs was used in the following experiments.

As the result of high *inferior vena caval ligation* (diameter down 60–80%), serum GOT activity rose in only one instance above the control operative limit of 200 units, to 670 units. Serum LD activity fell or rose insignificantly. Enzyme activities of the liver did not fall significantly.

Portal vein ligation (diameter down 50–90%) again was followed by insignificant changes in enzyme activities in the serum. Enzyme activities of the liver fell in some instances (with hepatic cell damage and decreased cholesterol content), but to a lesser degree than in the hepatic artery ligated dogs.

Complete *ligation* of the *common bile duct* proximal to the cystic duct resulted in maximal rises at 8 hours in serum GOT slightly above 200 units in 2 out of 8 dogs. Serum LD activity rose above 1000 units in 4 out of 8 dogs, maximally at 4 hours (2), 8 hours (1) and 1 week (1). The expected rises in serum bilirubin (direct), alkaline phosphatase, thymol turbidity (later), serum and liver cholesterol levels occurred generally. The hepatic enzyme activities were not decreased except for moderate falls in GOT activity in dogs showing marked bile stasis plus central hepatic cell degeneration.

The Kidney as a Source of Blood Ammonia in Resting and Hyperventilated Cirrhotics

By *J. Norman Berry, John F. Flanagan, Edward E. Owen and Malcolm P. Tyor.* Department of Medicine, Duke University Medical Center, and V. A. Hospital, Durham, North Carolina.

Patients with liver disease, whose exogenous ammonia source is negligible, may exhibit elevated arterial ammonia values. The present report deals with the relative contribution of liver, kidney and extremity to the blood ammonia concentration, with particular emphasis on the renal contribution during hyperventilation.

Nine cirrhotic patients were studied. Arterial and venous blood was obtained from a brachial artery and a catheterized hepatic, renal and iliac vein. Positive A-V (arterial-venous) ammonia differences indicated ammonia uptake; negative differences indicated ammonia release. In 5 patients renal A-V ammonia differences were obtained after 20 minutes of hyperventilation.

Arterial-venous ammonia differences across liver and extremity were primarily positive; mean = +30 and +31 μg./100 ml., respectively. In contrast, renal A-V ammonia differences were consistently negative; mean = −37 μg./100 ml. Arterial ammonia concentrations increased in 4 of 5 patients after 20 minutes of hyperventilation when compared with resting values; mean difference = +13.2 μg./100 ml., $p = <0.10$. Of particular import was the concomitant increase in renal vein ammonia concentration, resulting in augmentation of the negative A-V ammonia difference after hyperventilation; mean difference = −14 μg./100 ml., $p = <0.01$. These changes were observed in association with significant increases in arterial pH and variable but minimal changes in renal A-V oxygen differences.

These preliminary data suggest that the kidney may contribute significantly to the arterial

ammonia concentration of cirrhotic patients, and that this contribution may be accentuated by respiratory alkalosis. The data confirm the importance of the liver and extremity in reducing the blood ammonia.

The Toxicity of Peritoneal Fluid Resulting from Strangulation of the Gastrointestinal Tract at Various Levels

By *William O. Barnett*. Department of Surgery, University Medical Center, Jackson, Mississippi.

There is considerable evidence which indicates that bacteria play a major role in the lethal issue of strangulation obstruction. The bacterial flora of the upper gastrointestinal tract is relatively low in comparison to the ileum and colon. It was, therefore, elected to compare the relative toxicity of fluid resulting from strangulation of various segments.

Peritoneal fluid for this study was obtained by strangulation of the stomach (4 dogs), duodenum (2 dogs), jejunum (2 dogs), ileum (2 dogs) and colon (2 dogs). The accumulated peritoneal fluid was collected 36 hours after strangulation or at the time of death in those animals that failed to survive this period. Forty normal dogs were divided into 5 groups. Each group was given an intraperitoneal injection of fluid which resulted from strangulation of one of the segments of the gastrointestinal tract. Eight of 15 animals recovered after injection with fluid resulting from strangulation of the stomach. Four of 5 animals recovered after exposure to duodenal fluid. There were 3 out of 5 survivors when jejunal fluid was injected. There were no survivors following exposure to ileal and colon fluid (15 dogs).

Previous studies have shown that the degree of leukopenia following exposure to peritoneal fluid resulting from strangulation obstruction closely parallels the toxicity of this material. Four hours after injection of peritoneal fluid resulting from strangulation of the stomach, the mean WBC count was found to be 8,850/mm.^{3} Immediately before injection of the fluid this value was 11,165. Injection of colon fluid resulted in a drop in mean WBC count from 12,800 to 3,800.

It is concluded that the toxicity of peritoneal fluid resulting from strangulation increases from the upper to the lower gastrointestinal tract.

The Effects of Temporary Arterial, Venous and Arterio-Venous Occlusion on Intestinal Blood Flow

By *M. D. Turner, William A. Neely and William O. Barnett*. Departments of Surgery and Biochemistry, University of Mississippi School of Medicine, Jackson, Mississippi.

The effects of occlusion upon the vascular integrity of the bowel must be evaluated if we are to understand better the underlying mechanisms of certain gangrene-producing conditions.

Midgut segments were selected, and blood flow was measured according to the method of Neely and Turner. Control measurements were taken on each segment. Measurements were taken immediately after the release of the occluding clamp and 1 hour later. Approximately 10 animals were studied in each group.

In 9 animals in which the vein draining the segment was occluded for 30 minutes, the flow was significantly reduced immediately after release and remained so one hour later ($P < .005$).

In 10 animals in which the artery and vein to the segment were occluded for 1 hour, no significant changes in flow were noted immediately and 1 hour later.

In 10 animals in which the artery to the segments was occluded for 1 hour, blood flow was significantly reduced immediately and at one hour after release ($P < .01$).

Conclusion: Venous occlusion is most detrimental to intestinal blood flow. Arterial occlusion is also detrimental, whereas arteriorvenous occlusion had no effect. In the kidney, however, arteriorvenous occlusion was most detrimental, whereas arterial occlusion had little effect.

Resection of the Small Bowel as Prophylaxis in Hemorrhagic Shock

By *Watts R. Webb, S. S. Lee and John M. McRae, Jr.* Department of Surgery, University of Mississippi School of Medicine, Jackson, Mississippi.

Though pathologic changes have been noted many times following hemorrhagic shock, the precise role of the small bowel has not been delineated. Lillehei found that perfusion of the superior mesenteric artery presented these changes and protected against irreversible hemorrhagic shock more certainly than perfusion of the liver or other organs.

Dogs were maintained in hemorrhagic shock by bleeding into arterial reservoirs at 30 mm. Hg, with recordings being made of the times of reversal and of death. Similar observations were recorded after resection of the entire small bowel. The time of reversal in the 15 control dogs

averaged 1 hour and 36 minutes, while 18 experimental animals with the small bowel resected averaged 2 hours and 18 minutes ($P < .005$). The survival times of the control series averaged 5 hours and 40 minutes, compared to 7 hours and 15 minutes in the resected series. In addition, the resected animals lived much longer after complete uptake of the shed blood.

The resected bowel pre-shock averaged 368 Gm., while the bowel post-shock weighed over 100 Gm. more. In 6 additional control dogs in which the ileocecal junction was clamped prior to shock, the weight of the small bowel at death was found to average 596 Gm. Over 200 cc. of bloody fluid were present in the lumen of the bowel with the hemoglobin content averaging 7 Gm.%. In these animals there was very little bloody fluid in the stomach and less in the large bowel than in the small bowel. The mesenteric showed little engorgement, and microscopic changes were greatest in the small bowel.

These results indicate that loss of bloody fluid into the small bowel, which seems peculiarly sensitive to the hypovolemic state, appears to be one of the major contributing factors in the development of irreversibility in experimental hemorrhagic shock.

A Reticulocyte Response Following the Administration of Serine to Patients with Tropical Sprue in Relapse

By *C. E. Butterworth, Jr. and Enrique Perez-Santiago.* Department of Medicine, Medical College of Alabama, Birmingham, Alabama, and Department of Medicine, University of Puerto Rico School of Medicine, San Juan, Puerto Rico.

The concept that sprue is caused by a primary dietary deficiency of folic (FA) has been questioned by French and by others. The leukocyte content of FA has been found normal in certain patients with sprue in severe relapse. Furthermore, sprue patients are capable of converting glycine to serine, a process which requires FA. The single-carbon donor for this reaction appears to be N^{10} hydroxymethyl-tetrahydrofolic acid, which is derived from citrovorum factor (CF) through steps requiring ATP and TPNH. It seemed theoretically possible to reverse this reaction by administering serine, thus producing CF or other active compounds.

Accordingly, 7 Puerto Rican subjects with tropical sprue in relapse were given DL serine in oral doses of 5 Gm. twice daily for 7 to 13 days. Reticulocyte peaks of 5%, 6.2%, 6.8% and 7.4%, respectively, were observed in 4 patients temporally related to serine administration. Another patient showed a reticulocyte peak of 10.2% during serine therapy, but the result has been considered equivocal because he also received an oral dose of vitamin B_{12} 9 days earlier. Bacteriologic assays on urine specimens from 1 subject showed normal amounts of FA activity during serine administration.

The findings indicate that the enzymes and folic acid co-factors concerned in glycine-serine interconversions are capable of function at or near normal rates. They tend to implicate a metabolic disturbance involving other enzymes and/or the associated folic acid co-factors, as opposed to a primary deficiency of folic acid intake.

The Effect of Laxative-produced Diarrhea on Electrolyte Balance and Renal Function in Humans

By *Edward C. Wilson and James C. Respess.* University of Virginia Hospital, Charlottesville, Virginia.

The immediate and short-term effects of laxative-induced diarrhea on electrolyte balance and renal function were studied in 2 patients on the metabolic unit, using an aloin and cascara compound (Hinkle's Cascara).

The 1st patient had used Hinkle's for 12 years and was hospitalized with edema, abdominal distress and rectal stenosis. During an infusion of glucose, she developed a generalized flaccid paralysis and serum K of 1.9 mEq./L. Complete recovery followed electrolyte repletion and, on a subsequent balance study, she responded to 2 Hinkle's capsules per day by increasing daily fecal K from baseline of 15 mEq. to as high as 130 mEq., decreasing daily urine K from baseline of 72 mEq. progressively down to 9 mEq., and maintaining negative K balance which reached 66 mEq. at its lowest point. Stool volume was directly proportional to increase in fecal K. Renal function and biopsy were normal.

A healthy, normal female volunteer responded to 2 Hinkle's capsules per day by initially increasing daily fecal K from a baseline of 20 mEq. to 42 mEq. and decreasing daily urine K from 29 mEq. to 10 mEq., with a transient

negative K balance. Stool volume initially doubled but, with continuation of laxative, the stool volume, fecal and urine K returned toward normal. Renal function remained normal.

Renal biopsy on a 3rd patient, who had been treated 3 months previously for hypokalemia from Hinkle's induced diarrhea, showed changes suggestive of clear cell nephrosis. At the time of biopsy, serum electrolytes and renal function were normal.

Diarrhea resulting from Hinkle's produces prompt fecal loss of K which may be sustained in sensitive individuals producing chronic hypokalemia. The amount of fecal K lost is apparently directly proportional to the volume of feces. Changes in renal function were not seen as an immediate effect of laxative-induced diarrhea.

Studies of Hydrocortisone Absorption from the Colon of Patients with Idiopathic Ulcerative Colitis

By *Marcel Patterson and Raymond Gregory,* with the technical assistance of *Alene Bennett and Elizabeth Heidrick.* Department of Medicine, and John Sealy Memorial Laboratory for Clinical Research, University of Texas Medical Branch, Galveston.

Topical therapy with hydrocortisone or its analogs has proved efficacious in producing rapid symptomatic and proctoscopic remissions in over 75% of 27 patients with idiopathic ulcerative colitis. Similar symptomatic improvement has been observed in 17 of 20 patients with radiation proctitis, although the proctoscopic improvement has been much less striking. In an effort to detect the degree of systemic absorption, plasma hydrocortisone levels measured by the method of Silber and Busch, 24-hour urinary 17-ketosteroids measured by the method of Zimmerman et al., and 17-hydroxycorticoids measured by the method of Reedy et al. were studied in 5 patients with ulcerative colitis following 10 mg. or 50 mg. rectal instillations of hydrocortisone acetate and sodium succinate.

The plasma hydrocortisone rose to a mean of 2.5 μg.% (S.D. 4.6) at 1 hour, 2.9 μg.% (S.D. 6) at 2 hours and 6.5 μg.% (S.D. 4.4) at 4 hours in 3 patients. In 2 patients, the 24-hour urinary 17-ketosteroid excretion was not increased on the treatment day over the control day. In one of these patients, 24-hour urinary 17-hydroxycorticoid excretion was increased 3.2 μg.% on the treatment day.

These results further confirm that the degree of absorption of hydrocortisone from the colon in patients with idiopathic ulcerative colitis is very slight and suggest that the therapeutic benefit is primarily a local action. The obvious advantages of minimal absorption and rapid remissions in this disease are fewer side effects and the ability to use steroids in situations where this therapy might otherwise be contraindicated.

GENITAL TRACT

Estrogen and Progesterone Levels in Fetal and Maternal Plasma at Parturition

By *John R. K. Preedy, Elsie H. Aitken, Bruce Eton and Roger V. Short.* Department of Medicine, Emory University, Atlanta, Georgia, and Department of Veterinary Clinical Studies, University of Cambridge, England.

Although the cause of the onset of labor in the human is unknown, one theory suggests that labor is precipitated by a fall in circulating estrogens or progesterone. To test this theory, estrone, estradiol-17β, estriol and progesterone levels were determined in fetal and maternal plasma at elective Caesarean section (7 subjects) and at normal labor (8 subjects). Plasma estrogens were measured by the method of Preedy and Aitken (Lancet *1*:191, 1957) and plasma progesterone by the method of Short (J. Endocrin. *16*:415, 1958). Mean maternal plasma levels of estrone, estradiol-17β, estriol and progesterone at elective Caesarean section were respectively (μg./100 ml.) 26.6, 2.8, 24.4 and 13.0. At normal labor the corresponding values were 12.0, 2.2, 17.8 and 11.3. Mean fetal plasma levels of estrone, estradiol-17β, estriol and progesterone were respectively (μg./100 ml.) 2.15, 0.66, 140.8 and 38.5. At normal labor the corresponding values were 3.6, 0.5, 129.0 and 46.4. The differences observed between the two groups of subjects (Caesarean section and normal labor) in respect to any of

the hormones estimated were not significant. Consequently it appears that, in the human, the onset of labor is not associated with any significant alteration in the plasma levels of the three classical estrogens or of progesterone.

It was, however, observed that significant differences did exist between fetal and maternal plasma levels of all four hormones estimated. In particular the plasma concentration of estriol and of progesterone was markedly higher in fetal than in maternal plasma. Although the evidence is not yet complete, it is possible that a concentration gradient across the placenta exists in respect to estriol and progesterone, which may be of importance in any transfer of these hormones from fetal to maternal circulation.

INFECTIOUS DISEASES

The Effect of Bacterial Endotoxin on Adrenal Medullary Function

By *Richard H. Egdahl.* Department of Surgery, Medical College of Virginia, Richmond, Virginia.

Bacterial endotoxin and epinephrine have many similar effects when given systemically to animals. For this reason, it has been suggested that at least some of the effects due to bacterial endotoxin are mediated through epinephrine release. The purpose of this study was to determine the effect of bacterial endotoxin on adrenal medullary function. The lumbo-adrenal vein was cannulated in 19 normal dogs and 3 dogs with spinal transection at C7, so that intermittent collections of adrenal venous blood could be obtained. E-Coli endotoxin was given intravenously to chronic, unanesthetised, adrenal cannulated dogs, and the output per minute of epinephrine and norepinephrine from the right adrenal medulla was determined by the Aronow modification of the method of Weil-Malherbe and Bone. 17-Hydroxycorticosteroid determinations were carried out using the method of Nelson and Samuels.

When endotoxin was administered in a dosage of 0.01 mg., only an occasional normal animal showed an increased secretion of either epinephrine or norepinephrine, whereas all animals at this dosage level manifested both fever and significant 17-hydroxycorticosteroid response. When the dose of endotoxin was increased to 0.2 mg., all animals not only became febrile and had adrenal cortical responses, but in addition there was a significant increase in the adrenal output per minute of both epinephrine and norepinephrine. Cord-sectioned animals responded with the usual fever and adrenal cortical secretion increase following a dose of 0.2 mg. of endotoxin, but in contrast to the normal there was no catechol amine response.

These studies demonstrate that doses of bacterial endotoxin which are capable of eliciting fevers and increased adrenal cortical secretion may fail to give an adrenal medullary response. Large doses of endotoxin invariably resulted in increased adrenal medullary secretion. It appears clear from these studies that adrenal cortical response and fever resulting from endotoxin administration are not due to increased secretion of catechol amines from the adrenal medulla. Results of the cord section experiments indicate that afferent or efferent nervous pathways, or both, are important in the adrenal medullary response, and that endotoxin does not act directly on the adrenal medulla.

Penicillin "Fall-Out" in Relation to Nasal Carriers of Staphylococci

By *Jack A. Barnett, Robert E. Windom and Jay P. Sanford.* Department of Internal Medicine, University of Texas Southwestern Medical School, Dallas, Texas.

The incidence of coagulase positive *Staphylococcus aureus* in the anterior nares of persons in hospitals and in the community are comparable; however, penicillin-resistant strains predominate in hospitals, in contrast to a predominance of penicillin-sensitive staphylococci in the general population. This difference has been explained by postulating free interchange of organisms between patients and personnel. Gould demonstrated aerosols of penicillin in hospital environments. Deposition of penicillin in the nares would favor selection of resistant organisms.

To confirm the occurrence of penicillin "fall-out," Petri plates containing agar seeded with *Sarcina lutea* (A.T.C.C. No. 9341) were exposed in the hospital, medical school and private clinics. Control plates containing penicillinase were likewise exposed.

Particles of penicillin ranging from trace amounts to 0.4 units settled on plates exposed. The same phenomenon was demonstrated in two

private medical clinics, being extremely heavy in the "injection rooms."

The anterior nares of patients and personnel were studied for the presence of penicillin and strains of coagulase positive staphylococci. The staphylococci were studied for bacteriophage as well as penicillin susceptibility. Penicillin was demonstrated in the nares of 18% of 188 patients and personnel studied. Penicillin in the nares bore no relation to penicillinemia; only 5 of 20 patients receiving penicillin had penicillin in their nares, while 6 of 19 patients not receiving penicillin, but in rooms where penicillin was administered, demonstrated penicillin in their nares. Thus, penicillin in the nares seemed related to "fall-out."

No distinct correlation existed between the epidemic (80/81) strains of staphylococci and the presence of penicillin; however, strains of staphylococci isolated from persons with penicillin in their nares were generally resistant to penicillin.

These observations support the importance of penicillin "fall-out," with the nares functioning as a mobile air-sampling device, in the acquisition of penicillin-resistant staphylococci.

The Efficacy of a Simple Egg Yolk Test on Determining Phagetypability of Staphylococci

By *James B. Grogan and Curtis P. Artz.* Surgical Bacteriological Laboratory and the Department of Surgery, University of Mississippi School of Medicine, Jackson, Mississippi.

Phagetyping of staphylococci is becoming increasingly important. An appreciable number of cultures are not typable. Since the procedure is quite time consuming, there is great waste of effort if the strains are not typable. This would assume major significance should the proposed phagetyping centers be organized. A study of the efficacy of a simple egg yolk test in determining phagetypability was carried out.

Six hundred and twenty-four cultures of coagulase-positive staphylococci which were collected over a 3-year period were phagetyped as well as grown on a special egg yolk media. This media was 5% egg yolk in trypticase soy agar. The production of an opalescent zone around the colony on the egg yolk media after 24 hours incubation was correlated with phagetypability.

It was found that 88% of the 624 cultures were phagetypable, and that every one of the phagetypable cultures also produced an opalescent zone on egg yolk media. Only 6 of the 74 strains which were not phagetypable produced opalescent zones.

It seems that this is a very easy, accurate method of determining whether a particular strain of staphylococcus can be phagetyped.

Epidemiologic Studies of an Outbreak of Staphylococcal Disease

By *Harris D. Riley, Jr. and William Kilgore.* Department of Pediatrics, Children's Memorial Hospital, University of Oklahoma School of Medicine, Oklahoma City.

An opportunity presented which permitted observations concerning the spread of staphylococcal infection among hospital patients and personnel, between hospitals, and, to some degree, in families.

Two siblings were admitted with a 4-month history of mild staphylococcal infections, finally culminating in severe disease in both children. The mother was employed as a nurse in a hospital (Hospital S) in a neighboring town. Further investigation revealed that an outbreak of staphylococcal infection had occurred among patients and personnel at Hospital S shortly after she began her nursing duties there. The mother was found to be a nasal carrier of a coagulase-positive staphylococcus of the identical antimicrobial susceptibility pattern and bacteriophage type (81/70/44 A) as that one responsible for the infection in her 2 children.

An epidemiologic and cultural survey of personnel and patients related to the involved divisions of Hospital S was conducted. Nasal and other types of cultures as indicated were processed by routine bacteriologic and phagetyping methods.

Several individuals in the survey were found to have overt skin or other types of staphylococcal infection. Coagulase-positive staphylococcus were recovered from 43 to 59 hospital personnel and patients. The majority of strains were of the same phagetype as that isolated from the 2 index children and their mother.

An outbreak of staphylococcal infections occurred in the ward of another hospital to which an employee carrying this strain was transferred.

Available data suggest that the index children's mother who was a nasal carrier introduced the infection into both her own family group and the hospital where she was employed.

The Influence of Infection with a 1957 Influenza A Virus on Staphylococcal Infection of the Bronchopulmonary Tissues of Mice

By *Thomas F. Sellers, Jr., Jerome Schulman, Rob-*

ert McCune and Edwin D. Kilbourne. Emory University School of Medicine, Atlanta, Georgia, and Department of Public Health and Preventive Medicine, New York Hospital–Cornell Medical Center, New York City.

Normal mice and mice infected with 1957 influenza A virus were infected with staphylococci by aerosol or intranasal technics and by the intravenous route. Irrespective of the route of infection the staphylococci usually disappeared in the normal mouse lung in 2 to 3 days. In the presence of the influenza viral infection, however, staphylococci introduced through the respiratory tract were not removed except when the viral infection had evolved to a stage reached between the 7th and the 10th day. Thereafter, staphylococci were eliminated as rapidly from the virus-infected mice as from the control animals. Significant multiplication of the staphylococci did not occur in the bronchopulmonary tissues. The influenza viral infection had no apparent effect upon the over-all course of an intravenously introduced staphylococcal infection in the mouse. Differences in the fate of intravenously introduced and inhaled staphylococci in the bronchopulmonary tissues appeared to be related primarily to the presence of removal mechanisms in the bronchiolealveolar area which were inactivated by influenza viral infection. In a number of the animals that had received infective or noninfective material through the respiratory tract, *Pasteurella pseudo-tuberculosis* appeared in the bronchopulmonary tissues. In contrast to the staphylococci, the pasteurella showed the capacity to increase appreciably in the broncho-pulmonary tissues and at times were the only bacteria present in localized purulent lesions.

Factors Affecting Drug Resistance of Urinary Pathogens

By *S. E. Grossberg, R. G. Petersdorf, James A. Curtin and I. L. Bennett, Jr.* Departments of Medicine and Pathology, Johns Hopkins University School of Medicine, Baltimore.

It is well-known that hospital staphylococci are more resistant to antibiotics than non-hospital strains. This study was performed to determine whether organisms usually responsible for urinary tract infections demonstrate similar antimicrobial resistance.

All urinary pathogens isolated during a 1-month period were studied; 208 strains from 161 patients were available for analysis. Tube-dilution sensitivities with bacteriostatic and bactericidal endpoints were performed against 10 antibiotics, and each organism was classified as resistant or sensitive according to currently accepted criteria. Results were correlated with preexisting structural abnormality of the urinary tract, surgical manipulation, previous drug treatment and origin of the infection—home or hospital.

The gram-negative flora consisted of *E. coli* (77 strains), *Klebsiella* (29), *Proteus* (27), *Paracolon* (17) and *Pseudomonas* (10). There were 48 strains of streptococci and no staphylococci. Ninety-two % of streptococci, 81% of *E. coli* and 69% of *Klebsiella* were sensitive by the criteria used, but 53% of *Paracolon*, 81% of *Proteus* and 90% of *Pseudomonas* strains were resistant. Even among sensitive strains few antimicrobials demonstrated true bactericidal action.

Drug resistance was more than twice as common among *E. coli* and *Klebsiella* acquired in hospital than among non-nosocomial strains; most of the remaining gram-negative bacilli were resistant "hospital" organisms. There was no correlation between structural abnormalities of the urinary tract and antimicrobial resistance, although most diabetic patients even with apparently normal excretory systems harbored resistant organisms. Catheterization and cystoscopy were responsible for infections in many normal patients; ⅓ of these were caused by resistant organisms. Regardless of whether infection occurred in or out of the hospital, prior exposure to antibiotics enhanced antimicrobial resistance.

These data indicate that among the factors adversely affecting the antibiotic sensitivity of urinary pathogens are origin in hospital, prior exposure to antibiotics and, perhaps, diabetes mellitus.

The Ear Lobe Phagocytic Monocyte in Bacterial Endocarditis

By *Lamar Crevasse.* (Gainesville, Florida.) Department of Medicine, Emory University School of Medicine, Georgia.

Large numbers of phagocytic monocytes have been noted localized to the ear lobe blood in patients with S.B.E., and this has been emphasized as an important finding in diagnosis. To determine the incidence, specificity and relationship of this cell for S.B.E., a 200-cell differential on the 1st and 2nd drops of ear lobe blood and on the 1st drop of finger puncture blood and a total W.B.C. on the 3rd drop of ear lobe blood and the 2nd drop of finger puncture blood were determined. These were performed on 3 consecu-

tive days in the following groups of untreated consecutive patients: S.B.E.—8, all bacteriologically proven; lupus erythematosus—5; acute rheumatic fever with carditis—7; tuberculosis—3; Hodgkin's disease—3; periarteritis nodosa—1; and 10 normal adults.

The ear lobe total W.B.C. exceeded the simultaneous finger puncture total W.B.C. in 178 of 188 determinations, including all groups studied. The widest range was 3400/mm.^{3} for the finger and 12,800/mm.^{3} in the simultaneous ear lobe puncture. These differences were significant, $P < .01$. There was, however, no difference between diseases.

Only one patient with S.B.E. had a substantial increase in ear lobe phagocytic monocytes (14%); the remainder of this group averaged 0.87% for finger and 4.4% for the ear lobe determination. These cells were present in both finger and ear lobe blood in 6 of 7 patients with acute rheumatic fever (0.5–1.5%; 6 of 10 normals, 0.5–5.5%); 4 of 5 with L.E. (0.5–5.5%; and 6 of 7 with other diseases, 0.5–8.5%). The percentage phagocytic monocytes was significantly higher only in the ear lobe blood of the group with S.B.E. ($P < .01$). No significant difference was found between finger and ear lobe or percentile differences in the ear lobe per se in the other diseases. The phagocytic monocyte has no specificity for bacterial endocarditis; it is present in normal humans and in varying degrees of every disease state as a nonspecific response to stimulation of the reticuloendothelial system. There was no correlation with duration of illness or type of organism. The minor satistical differences are of no clinical usefulness in the diagnosis of S.B.E.

Purification of Soluble Antigens of Histoplasma Capsulatum by means of Electrophoresis and Agar Diffusion Technics

By *Douglas C. Heiner, Eugene J. Towbin and Arliss H. Tuttle.* Departments of Pediatrics and Medicine, University of Arkansas Medical Center and V. A. Hospital, Little Rock, Arkansas, and Department of Pediatrics, University of Tennessee Medical School, Memphis, Tennessee.

The diagnosis of histoplasmosis has always been difficult to make because it is seldom possible to isolate the causative organism except in the infrequent instances of chronic pulmonary or disseminated histoplasmosis. A single positive skin test may be used only as evidence of past infection. In spite of a good antibody response in patients with the disease, many attempts at serologic diagnosis are unsuccessful because of frequent cross reactions, particularly to antigens of Blastomyces dermatitidis and Coccidioides immitis. It has been shown that this problem may be overcome by using Ouchterlony's double diffusion precipitation technic in an agar gel medium. The major disadvantage of the technic when applied to clinical usage has been the need for experience in identifying the different antigen-antibody systems which are encountered. Without identification of individual antibodies the test is superior in several respects to conventional precipitin tests. However, with specific antibody identification the test is considerably more reliable than any currently available immunologic test for histoplasmosis.

It has been possible to separate the 3 important soluble antigens of Histoplasma capsulatum from one another by means of continuous flow paper electrophoresis. Using purified antigens prepared in this manner it is possible to identify specific precipitins in agar gel without the need for control sera of known antibody content or for extensive experience with the technic. The test may readily be performed on single or multiple serum specimens.

KIDNEY

Effect of Chronic Hypercalciuria on Renal Conservation of Sodium and Water

By *John R. Gill, Jr. and Frederic C. Bartter.* Section of Clinical Endocrinology, National Heart Institute, National Institutes of Health, Bethesda, Maryland.

Maximum urine osmolality, T^cH_2O, and sodium clearance were measured in idiopathic hypercalciuria, sarcoidosis, hyperparathyroidism and hypoparathyroidism over-treated with vitamin D. Renal function, estimated from BUN, PSP and GFR, was intact. Maximum osmolality was determined after a 24-hour thirst, during pitressin infusion (200 mU./hr.); T^cH_2O was determined during osmotic diuresis produced by 10% mannitol, 18 cc./min., intravenously, containing pitressin (200 mU./hr.), following the protocol of

Zak, Brun and Smith (J.Clin.Invest. 33:1064, 1954). In all conditions studied, urinary calcium was varied from high to normal by changing dietary calcium and giving oral Disodium Versenate. With increased urinary calcium, maximum osmolality, the more sensitive index, decreased from a mean of 800 mOsml. to 560 mOsml.; T^cH_2O decreased finally from a mean of 5.4 cc./min. to 3.2 cc./min. In sarcoidosis and idiopathic hypercalciuria one could, by lowering urinary calcium to normal, improve maximum osmolality and T^cH_2O (558 mOsml. to 800 mOsml. and 3.2 cc./min. to 5.5 cc./min., respectively). T^cH_2O improved first. In a vitamin D-treated patient with hypoparathyroidism, maximum osmolality and T^cH_2O returned from 372 mOsml. and 0.1 cc./min., respectively, to normal (838 mOsml., 5.3 cc./min.) on stopping vitamin D alone, although hypercalciuria continued. In another patient, when vitamin D was given in large doses together with Versenate, concentrating ability remained impaired (760 mOsml.) and T^cH_2O deteriorated (5.0 cc./min. to 3.2 cc./min.) despite persistently normal urinary calcium. Similarly, a patient with hyperparathyroidism and depressed concentrating power not only showed no improvement, but rather deterioration of T^cH_2O from 3.8 cc./min. to 1.0 cc./min., while Versenate maintained urinary calcium below 130 mg./day. Both parameters improved (545 mOsml. to 735 mOsml. and 1.0 cc./min. to 4.5 cc./min.) upon removal of a parathyroid adenoma. Although many patients showed at some time elevated serum calciums, this was not a prerequisite for the concentrating defect. All patients studied conserved sodium on a 9 mEq. sodium diet.

Thus, hypercalciuria was shown to produce reversible impairment of the kidney's ability to concentrate. Further, the data suggest that parathyroid hormone and vitamin D may not only produce a severer defect in concentrating power than hypercalciuria alone, but also may affect the mechanism by means other than hypercalciuria.

The Influence of Various Diuretic Agents on the Urinary Excretion of Magnesium in Non-Edematous Subjects

By *William O. Smith, Adrian Kyriakopoulos, David C. Mock and James F. Hammarsten.* Medical and Radioisotope Services, V. A. Hospital, Oklahoma City.

The magnesium deficiency syndrome has been observed in 2 patients with congestive heart failure following vigorous diuresis induced by a mercurial diuretic. Although the medical literature contains numerous reports concerning the effect of various diuretic agents on the urinary excretion of commonly measured electrolytes, no such observations have been reported in regard to magnesium, with the sole exception of one series of 10 patients with congestive heart failure given Mercuhydrin. The present study is concerned with urinary magnesium excretion following the administration of mercaptomerin, chlorothiazide, acetazolamide and a placebo.

Non-edematous subjects were studied while on a metabolic balance regimen. Following a 2-day control period, the test drug was administered for 2 days. Complete electrolyte studies of the urine, including magnesium, were made. Urinary magnesium determinations were performed by spectrophotometric titration with EDTA after removal of calcium by ammonium oxalate. This method is accurate within 3% in recovery studies.

Nine patients were given 2 cc. daily of mercaptomerin. A mean increase in urinary magnesium excretion of 30% above control values was noted, with individual increases ranging from 4% to 52%. The increase is statistically significant ($P < .05$). Eight subjects received 1500 mg. of chlorothiazide by mouth daily. Individual increases in urinary magnesium excretion ranged from 5% to 52%, with a mean increase of 33%. This increase is significant ($P < .05$). Only 4 patients have received acetazolamide to date. Thus far, it appears that this agent has little effect on magnesium excretion. Five subjects receiving the placebo showed no significant change in urinary excretion of magnesium.

Significant urinary losses of magnesium may be occasioned by the administration of mercurial diuretics and chlorothiazide, even in non-edematous subjects.

Renal Excretion of Magnesium and the Renal Factors Responsible for Hypermagnesemia of Renal Disease

By *Roscoe R. Robinson, Herschel V. Murdaugh, Jr. and Ernst Peschel.* Departments of Medicine, Lackland Air Force Base, University of Alabama, Birmingham V. A. Hospital, and Duke University.

To define the renal patterns of magnesium excretion in renal disease simultaneous determi-

nations of inulin and magnesium clearances were performed in 20 patients with different types and degrees of renal disease. In some instances the ultrafiltrable magnesium was measured. Subjects were maintained on regular hospital diets or diets restricted in salt and/or protein. No patients received increased magnesium intake or drugs previously known to alter magnesium excretion.

A definite relationship was found between total serum magnesium concentration and the degree of reduction of GFR (C_{In}). A normal serum magnesium concentration was found in each patient whose GFR was greater than 30 ml./min. Serum magnesium concentration was elevated in all subjects with GFR less than 30 ml./min., with but one exception. This patient, whose serum magnesium was normal despite a GFR of 14 ml./min., had received chlorothiazide daily. Studies of the effect of oral chlorothiazide in normal subjects showed that the urinary excretion of magnesium may be increased 7-fold by chlorothiazide.

Analysis of magnesium clearance data demonstrated that as the GFR decreased and total serum magnesium increased, the ratio C_{Mg}/C_{In} rose, while C_{Mg} and UV_{Mg} remained within normal limits. Some patients with elevated serum magnesium had an increased fraction of protein-bound magnesium. These findings indicated that magnesium excretion in renal disease is not proportional to the apparent filtered load, and suggest the presence of a compensatory mechanism to maintain the renal excretion of magnesium in advanced renal failure. In an attempt to evaluate such compensatory mechanisms, studies of magnesium excretion by stop-flow analysis were performed on dogs using magnesium-28. The Mg^{28} was injected with the inulin and PAH during the ureteral occlusion. Mg^{28} appeared in the collections from the distal nephron, demonstrating that non-filtered magnesium can enter the filtrate via the tubule.

The Effect of Arterial, Venous and Arterio-Venous Occlusion on Renal Blood Flow

By *William A. Neely and M. D. Turner.* Departments of Surgery and Biochemistry, University of Mississippi School of Medicine, Jackson, Mississippi.

This study deals with the effect of temporary circulatory stasis upon renal blood flow in the dog. This organ was selected in view of the fact that many surgical procedures upon the aorta result in temporary interruption of renal blood flow.

Adult mongrel dogs were anesthetized with pentobarbital and midline laparotomy incisions made. The left renal artery and vein were dissected free from all tissues throughout their entire length. Blood flows were measured according to the method of Neely and Turner. After control measurements had been obtained, the renal artery alone was occluded for one hour in one group. In another group the renal artery and vein were occluded for one hour. In another group the renal vein was occluded for 30 minutes. Flow measurements were made immediately after release of the clamp and one hour later.

In 10 animals whose renal vein was occluded for 30 minutes, the blood flow was significantly depressed immediately after release of the venous clamp and 1 hour later ($P < .05$).

In 11 animals whose renal artery and vein were occluded for 1 hour, the blood flow was significantly reduced immediately and 1 hour after release of the clamp ($P < .005$).

In 10 animals whose renal artery was occluded for 1 hour, the blood flow was significantly reduced ($P < .005$) immediately after release of the clamp but was not significantly reduced after 1 hour.

Conclusion: Occlusion of the renal vein is quite detrimental to blood flow for a short time after release of the vein. Occlusion of the renal artery and vein is also quite detrimental for a short period of time; whereas, occlusion of the renal artery is least detrimental to flow after a short recovery period.

Alterations in Renal Hemodynamics during Surgical Resection of Abdominal Aortic Aneurysms

By *William B. Berry, George C. Morris, Jr. and Michael E. De Bakey.* Cora and Webb Mading Department of Surgery, Baylor University College of Medicine, and Surgical Services of Methodist and V. A. Hospitals, Houston, Texas.

Recent investigations report renal damage subsequent to aortic surgery which may follow reflex vasoconstriction resulting from irritation of the sympathetic component of the autonomic nervous system around the abdominal aorta. This study was undertaken in an attempt to elucidate the etiology of the suppression of renal function which occasionally follows resection of abdominal aortic aneurysms.

Thirteen patients with abdominal aortic aneurysms arising distal to the renal arteries were used in this study. Observations of blood pressure, G.F.R. (glomerular filtration rate) and R.B.F. (renal blood flow) were made in each case. Control studies were performed the day before operation. During the operative procedure, observations were made during the period of anesthesia and laparotomy before occlusion of the aorta, during the period of aortic occlusion and after the release of the aortic clamp. Follow-up studies were performed 7 to 14 days after operation.

Average age for this group was 57 years with a range of 45 to 80. Aortic occlusion averaged 45 minutes. R.B.F. was reduced to 46% of control values during the period of anesthesia and laparotomy with no further alteration during aortic occlusion or following release of the aortic clamp. For the same respective periods, G.F.R. and water excretion were similarly depressed without further depression during the period of aortic occlusion. Studies made 7 to 14 days after operation correlate closely with control data, indicating complete return to normal renal function. In spite of the established relationship between functional capacity of the kidney and the sympathetic nervous system, it appears from this study that surgical manipulation of the aorta and adjacent sympathetics, together with aortic occlusion below the renal arteries, produces no depression in renal function.

Circulating Anti-Human Kidney Antibodies in Renal Disease

By *Norman C. Kramer, Mary F. Watt and Alvin E. Parrish*. Department of Medicine, George Washington University, School of Medicine, Washington, D.C.

The presence of antibodies to human kidney in the blood of patients with certain renal diseases has already been reported. The present study attempts to determine their usefulness in the diagnosis of glomerulonephritis.

An agglutination reaction between colloidal latex adsorbed human kidney extract and the serum of patients with symptomatic renal disease was used. A total of 36 sera from 28 patients with biopsy and/or clinical evidence of renal disease were examined for the presence of agglutinating antibody. In addition a rabbit was sensitized with a 10% extract of human kidney, and it exhibited the development of agglutinating antibodies. Agglutination in a dilution of 1:20 or more was considered as a positive titer. Seven of the samples tested revealed a significant and titer including: 3 patients with clinical evidence of glomerulitis; 2 of 9 patients with acute glomerulonephritis; 1 patient with chronic glomerulonephritis and the nephrotic syndrome; and 1 patient with intercapillary glomerulosclerosis. The 2 patients with glomerulonephritis exhibiting positive titers were tested within 3 weeks of onset of symptoms, while the 7 patients with negative titers were tested 1 to 2 months after the onset of symptoms. Four patients with disseminated lupus erythematosus were all found to have negative sera.

In conclusion, the data thus far obtained (1) confirm the presence of a circulating autoagglutinin to human kidney in patients with renal disease; (2) the autoagglutinin seems to be present in patients with active glomerulitis, offering an aid to diagnosis early in the course of the disease; (3) a new technic for demonstrating autoagglutinins to human kidney is presented.

The Nephrotic Syndrome, as Pertaining to the Clinicopathologic Correlation

By *Eugene J. Spiotta, A. L. Drerup and George Lumb*. Department of Medicine and Pathology, University of Tennessee, and John Gaston Hospital, Memphis, Tennessee.

This study was undertaken to determine the value of renal biopsy in the nephrotic syndrome, especially pertaining to the clinicopathologic correlation and towards predicting the response to steroid treatment.

Clinical investigative studies were performed using the conventional laboratory studies available to most hospitals. Needle biopsies were performed on one or both kidneys. Serial biopsies were performed whenever possible.

Fourteen cases were studied and included 2 cases of Kimmelstiel-Wilson syndrome, 3 of glomerulonephritis, 3 of arteriolonephrosclerosis, 2 of tubular degeneration without glomerular lesions, 3 of membranous glomerulonephritis and 1 of pyelonephritis.

It was found that the pathologic diagnosis seldom correlated with the clinical diagnosis. Response to steroid treatment could be predicted reasonably well by studying the pathologic specimen. Prognosis could be predicted in some cases.

Several of the pathologic findings noted revealed conditions uncommonly associated with the nephrotic syndrome. It is possible that the patho-

logic findings did not necessarily indicate the primary pathophysiologic mechanism responsible for this state.

The nephrotic syndrome can be produced by a multiplicity of causes, both glomerular and/or tubular. While renal biopsy has not provided all the answers regarding pathogenetic mechanisms at this time, it remains the most significant advance in the study of this state, and should be a part of the investigative evaluation of each nephrotic. Renal biopsy can best predict etiology, and response to treatment, as well as prognosis in many instances.

The Nature of the Hepato-Renal Syndrome: a Biochemical Study

By *James C. Graham and Louis Sulya.* Department of Biochemistry, University of Mississippi Medical Center, Jackson.

The nature—in fact, even the existence—of an "hepato-renal syndrome" has been long disputed. While studying NH_3 metabolism in Eck-fistula non-Dalmatian dogs, we noted that they excreted large amounts of uric acid—as does the Dalmatian dog who has a limited renal ability to reabsorb uric acid from the filtrate. Thus, it seemed obvious that some impairment had occurred either in the kidney or liver of the Eck-fistula dogs to allow uric acid to escape (uric acid is normally converted by uricase to allantoin in the liver). This study was designed to identify whether the functional lesion was renal or hepatic.

Five apparently healthy mongrel dogs were used. Clearance tests for uric acid, creatinine, glucose and allantoin were performed before and at intervals following Eck-fistulas.

Both uric acid and allantoin clearance were decreased in the Eck-fistula dogs, indicating a decreased glomerular filtration rate. In contrast, uric acid clearance was greatly increased, especially in the Eck-fistula dog surviving 1 year. All liver function tests except BSP (slightly elevated) were normal.

Conclusions: The data indicate a decreased glomerular filtration rate and a decreased tubular reabsorption in the Eck-fistula dog. Since blood uric acid levels failed to rise postoperatively while uric acid excretion increased, the defect appears to be renal and not hepatic.

Renal Tubular Acidosis and a Syndrome of Muscle Paralysis and Hypokalemia

By *Edward E. Owen and John V. Verner, Jr.* Medical Service, V. A. Hospital, and Department of Medicine, Duke University Medical Center, Durham, North Carolina.

Nine female patients presenting with flaccid quadriplegia and hypokalemia were found to have renal tubular acidosis with hyperchloremic acidosis and persistently alkaline urines. Balance studies in several demonstrated large negative potassium balances with excessive urinary excretion even during the hypokalemic phase.

Other common features included polyuria, polydipsia, hyposthenuria, nephrocalcinosis and pyelonephritis with occasional findings during the hypokalemic phase of normocalcemic tetany and hyperdynamic circulation.

Of possible etiologic significance was the presence of or history of pyelonephritis in 7 patients. The family history was negative in all, as were blood and urine analysis in 2 families studied. Histamine achlorhydria in 1 patient suggested the possibility of a generalized carbonic anhydrase deficiency, but her red blood cells contained normal activity of this enzyme (method of Miller et al.) when compared with 8 controls. The arterial-alveolar pCO_2 gradient was normal before and after exercise. These data do not suggest a generalized carbonic anhydrase deficiency, but do not exclude a localized deficiency in the kidney.

Each patient responded well to potassium and alkali therapy with no further paralytic episodes during a follow-up of 5 years in 6 patients. There has been no deterioration of renal function as tested by blood NPN and PSP excretion, although 5 have nephrocalcinosis and all have hyposthenuria. The only complication of therapy was transient hyperkalemia in 1 patient with fixed urinary potassium excretion, demonstrating the need for caution in potassium replacement. All patients have remained asymptomatic; 3 require no further potassium supplements; 1 has had an apparent remission; and the remainder require potassium and alkali therapy.

Patients presenting with muscle paralysis and hypokalemia may have renal tubular acidosis which will respond well to prompt diagnosis and therapy.

NEOPLASTIC DISEASE

Differential Behavior of Sera from Normal and Cancer Patients

By *Anwar A. Hakim*. Biochemistry Laboratories, Miami Heart Institute, Miami Beach, and Biochemistry Department, School of Medicine, University of Miami, Coral Gables, Florida.

Immunologic differences between malignant and normal cases will contribute to the etiology of cancer and its treatment. Studies on differential behavior of sera from persons with and without cancer are reported here.

Crystalline, chemically pure tumor phospholipids used in these investigations were obtained from adenocarcinoma of rectum, right breast tumors and left breast tumors.

Sera from cancer patients with known malignancies, with pleomorphic cancer of the lung, with renal cell carcinoma, with squamous cell cancer of the bronchus, with cancer of the breast and from normal healthy persons were used.

Hemagglutination reactions, similar to Kahn reaction in principle, were carried out on paper electrophoresis, the tumor phospholipids as the antigen and the sera as possible source of antibodies.

The efficiency of the different hemagglutination technics is compared with our own technic using paper electrophoresis. The antigenicity of tumor phospholipids obtained from human tumors is shown by increased precipitin titers and electrophoretic antigen-antibody interaction on paper electrophoresis of the sera from cancer patients.

Block titration revealed a phospholipid substance common to the types of tumors studied. Cross-reactivity, in precipitin reaction, between various human tumors was evident. Regular concentrates of the antigen showed no reaction with normal sera, while characteristic reaction patterns were produced with sera of cancer patients.

Two adult white rabbits of either sex, weighing between 3 and 4 Kg., were injected in the marginal ear vein with the tumor phospholipid solution obtained from human breast tumors. Two control rabbits were injected with the solvent solution in the absence of the tumor phospholipid. Injections were done twice weekly for a period of 3 months. Five ml. of blood were collected from each rabbit previous to every injection. The antigenecity of the tumor phospholipids from human breast tumors is confirmed by increased precipitin titers in the sera of animals injected with the phospholipid material from human tumors.

Recent hypotheses relate malignancy to the loss of cellular components which are essential to the normal control of cell multiplication. Our results indicate that tumors from cancer patients contain phospholipid materials that are responsible for the immunologic reactions of cancer sera. These components are absent from normal tissues, since sera of normal persons failed to react with the tumor phospholipids.

NERVOUS SYSTEM

Effect of the Drug Quiactin on Tracking and Complex Reaction Time

By *Calvin McFarling, Karl U. Smith and F. E. Shideman*. Departments of Psychology and Pharmacology and Toxicology, University of Wisconsin.

The present study was designed to test the effect of the drug Quiactin on the component movements in tracking and on complex reaction time of human subjects. Subjects were first trained for 5 days in order to minimize learning effects during the experiment. Twelve subjects (16 male and 6 female) were used in an experimental design which assigned 2 levels of the drug (400 mg. and 800 mg.) and a placebo to each subject to control for subject variability. The design called for all 6 possible orders of administration of the 2 dosage levels of the drug and placebo to control for learning effects. Analysis of the data showed that Quiactin does not affect reaction time. Analysis of the error scores in tracking showed that this also was unaffected by Quiactin. A wavelength analysis was performed upon the tracking records. Oscillations in the tracking record below 5 mm. were interpreted as tremor; those between 5 mm. and 10 mm. were interpreted as corrective movements; and those

greater than 10 mm. were interpreted as positioning movements. This analysis showed that Quiactin differentially affected tremor, causing some subjects to show more of the small oscillations under the influence of the drug and some to show less. It is suggested that this wavelength analysis provides a method for the exploitation and development of multivariate analysis technics in connection with their practical application in the study of drug effects.

Globus Pallidus Coagulation Technic

By *Orlando J. Andy and James S. Browne.* University of Mississippi Medical Center, Division of Neurosurgery, Department of Surgery, Jackson, Mississippi.

A simplified steriotaxic instrument has been constructed for the coagulation of the globus pallidus. The instrument is approximately 3½ inches in diameter and 2 inches in height. It is so constructed that the patient is allowed to move his head freely during the procedure of stimulation, recording and coagulation. The instrument can be readily screwed into a standard size burr hole. A bipolar electrode is placed through a universal joint located in the center of the instrument and directed to the pre-determined point of coagulation.

A system of coordinates was devised, based upon a vertical line placed between the anterior commissure and the posterior third of the sella. This line is considered frontal plane 25 in accord with the atlas of Speigle and Wycis. A line at right angles to that, running through the anterior commissure, is considered horizontal zero.

The location of the anterior commissure as a reference point is based upon the ventriculogram which is performed at the time of operation. Since anatomic relationships are not 100% constant from patient to patient, bipolar and unipolar electrical stimulation and electroencephalographic recordings are utilized in order to assist in determining desired locus for the coagulation.

Synthesis of Cholesterol and Fatty Acids in Peripheral Nerve

By *Ann H. Hughes and Sven G. Eliasson.* Department of Internal Medicine, University of Texas Southwestern Medical School, Dallas, Texas.

The connective tissue elements of peripheral nerves have usually been regarded merely as supportive structures for the nerve fibers. We have attempted to determine whether such connective tissue plays an active metabolic role in maintaining the function of the nerve. In particular, the function of this tissue in the synthesis of fatty acids and cholesterol has been studied.

Normal cat sciatic nerves were excised and the connective tissue elements of the nerve were then separated from the neural elements by gentle homogenization in a Dounce homogenizer. This procedure yields an homogenate of the nervous tissue which is now almost devoid of desoxyribonucleic acid, indicating that it contains nerve axons and myelin but no ruptured connective tissue cells. The connective tissue, on the other hand, can be isolated as a single fibrous structure. In this manner, the connective tissue and nerve proper can be separated and studied as discreet units.

Both intact nerves and nerves fractionated in the above manner were incubated with acetate-1-C^{14}, and the extent of incorporation of C^{14} into CO_2 cholesterol and fatty acids was determined.

The most striking finding in these studies is that almost all of the cholesterol synthesis took place in the fibrous connective tissue sheath. On the other hand, the nervous tissue itself was found to synthesize fatty acids but very little cholesterol. Recombination of the fractions did not augment the incorporation of acetate-C^{14} into either of these lipids. It would appear, therefore, that the connective tissue elements of nerves play an important function both in maintaining the normal integrity as well as in regeneration of peripheral nerves, in that these studies would indicate that the nerve is primarily dependent upon its connective tissue sheath as a source for its structural cholesterol.

An "Epidemic" of Chemical Meningitis

By *Winfrey W. Goldman, Jr. and Jay P. Sanford.* Department of Internal Medicine, University of Texas Southwestern Medical School, Dallas, Texas.

The development of acute aseptic meningitis after intrathecal injections, using equipment contaminated by traces of common chemical agents, is not well appreciated.

Recently, severe meningitis was observed in 5 patients who had received spinal anesthesia at delivery. Four patients were delivered with low

spinal anesthesia (4 mg. Pontocaine), the 5th by Caesarian hysterectomy with continuous spinal anesthetic by catheter (3.3% procaine).

None had symptoms or signs of febrile illness before delivery, but all developed fever, headache and meningeal signs associated with a leukocytosis within 4 to 14 hours following intrathecal injection. Although the cerebrospinal fluid findings were those of an acute bacterial meningitis, bacterial infection was excluded by failure to demonstrate organisms either on stained smear or bacterial culture.

No neurologic deficits were encountered and recovery was complete, usually within 24 hours. All patients received antibiotic therapy.

The literature suggests 2 distinct chemical meningitis syndromes. An insidious, progressive and sometimes lethal chronic arachnoiditis has been described and is usually associated with the intrathecal injection of detergents. Acute, transient meningitis, as seen in our patients, has been attributed to the intrathecal injection of phenols.

During the 11-month period in which our cases occurred, no agent other than distilled water was to be used in cleaning spinal anesthetic syringes and needles. However, it was likely that spinal trays prepared by a previous technic were available initially, and these may have contained syringes which had been immersed in phenolic antiseptic solutions (Amphyl), dried and autoclaved. It was also possible that irregularities in the newer technic permitted occasional syringe contamination.

It is suggested that previously reported complications following the intrathecal injections of various drugs, including antibiotics, may be the result of similar equipment contamination.

Cerebrospinal Fluid in Jaundiced Patients

By *John T. Galambos and Donald G. Rosenberg.* Medical Service, Grady Memorial Hospital, and Section of Gastroenterology, Department of Medicine, Emory University School of Medicine, Atlanta, Georgia.

Sixty-two, consecutive, unselected adult patients admitted with jaundice to Grady Hospital were studied. Simultaneous cerebrospinal fluid (CSF) and serum bilirubin, protein, sugar, WBC and differential, and VDRL were determined.

The patients were grouped in I: viral hepatitis (VH), 20 patients; II: leptospirosis (Lp.), 3 patients, and chlorpropamide jaundice, 2 patients; III: cirrhosis (c), 14 patients, Hodgkin's granuloma of liver (Hg), 3 patients; IV: biliary obstruction (BO), 11 patients; and V: hemolytic anemia, 1 patient, pneumonia treated with Novobiocin, 1 patient, diagnosis not proven, 7 patients.

Yellow or green CSF was seen in 4 of 20 with VH; three of 3 with Lp., 2 of 3 with Hg and 5 of 14 with C; 4 of 11 BO, and in the patient on Vancomycin therapy. CSF protein was 40 mg.% or more in 5 with VH, in 1 with Lp. and 3 with C (all 3 had CNS symptoms). Pleocytosis of CSF was found only in 3 of 3 Lp. patients. Polymorphonuclear (PMN) leukocytes were present in CSF of 4 of 20 with VH, 3 of 3 with Lp and in 1 of 11 with BO. The CSF sugar was low in 1 with VH and 2 with Hg.

There was a significant correlation between serum and CSF direct bilirubin in the group with VH ($r' = 0.5682$, $d' = 874$, $N = 23$). In a group of 17 with C and Hg ($r' = 0.7668$, $d' = 228$, $N = 18$) the correlation was not significant in 11 cases with BO ($r' = 0.3818$, $d' = 102$, $N = 10$). There was no significant relationship between duration of jaundice and CSF bilirubin, nor between CSF bilirubin and protein. The means of the CSF/serum bilirubin ratios showed no significant difference between the groups with VH, C and Hg, and BO. Both direct and indirect bilirubin was present in CSF regardless of the etiology of jaundice.

The yellow color of CSF is not directly related to CSF bilirubin levels. The yellow color of CSF cannot be used in the differential diagnosis of jaundice; however, if pleocytosis is also present, Lp. should be strongly suspected. Viral hepatitis may be associated with CSF changes suggesting meningeal pathology. In cirrhosis, abnormal neurologic finding and increased CSF protein with or without xanthochromic CSF usually is due to portal-systemic encephalopathy.

PHARMACOLOGY AND THERAPEUTICS

Experimental Study of the Metabolic and Physiologic Effects of Toxic Doses of Salicylates

By *Hooshang Guilak, Harry Lipscomb, John Scoggins and Harold L. Dobson.* Metabolic Section–Department of Internal Medicine, Baylor University College of Medicine, Houston, Texas.

There is considerable confusion concerning the effects of large doses of salicylates. Hyperglycemia, as well as hypoglycemia, has been reported. There is also considerable confusion concerning ketone production. In addition, a variety of defects in intermediary metabolism have been proposed. Part of the confusion has arisen because of failure to recognize that salicylate intoxication is divided into 3 phases: initial, intermediate and late. The initial phase has been investigated in 20 dogs who received sodium salicylate in the dose of 320 mg./Kg. while under light pentobarbital anesthesia. One group of dogs received no further therapy, a 2nd group received 1700 cc. of saline i.v., while a 3rd group received 1000 cc. 5% glucose in water i.v. In the 1st group of animals, the arterial blood sugar fell to an average level of 18 mg.%, CO_2 content to 32 vol.%; blood acetone and serum citrate were unchanged at the end of 3 hours. At this time all animals died of ventricular fibrillation. In the saline-treated animals the blood sugar did not drop as far, and the animals survived until sacrificed at 6 hours. In the glucose-treated animals, the blood sugar was slightly elevated until the i.v. was discontinued, then the level fell to 60 mg.% at 6 hours. Approximately 50% of these animals died between the 5th and 6th hour of ventricular fibrillation. Under the conditions of these experiments, it is apparent that the salicylates are hypoglycemic, do not cause ketosis and are cardiotoxic.

An Experimental Appraisal of Heparin in Burns

By *Byron E. Green and Curtis P. Artz.* Department of Surgery, University of Mississippi School of Medicine, Jackson, Mississippi.

There is abundant experimental evidence that sludging occurs after burning. In addition, many pathologic studies have demonstrated that multiple thrombi develop beneath the burned area. To test the hypothesis that heparin might have a beneficial effect in burns, 2 areas were selected for study: (a) the local lesion in burned rats and (b) the kidney in burned dogs.

The backs of white rats were clipped and given a standard non-lethal full-thickness burn. The animals were then divided into 3 groups: (a) no treatment, (b) heparin for 7 days beginning 4 hours post-burn, and (c) heparin for 7 days beginning 24 hours post-burn. Frequent, standardized color photographs and daily biopsies were taken of the burned areas. No adverse effects of heparin were seen. In the group heparinized 4 hours post-burn there was a marked decrease in healing time and smaller scars. The lesions in this group, in contradistinction to the other 2 groups, showed definite islands of viable epithelium and a broad peripheral zone of healing after 5 days. The microscopic sections showed blood vessels extending into the lesions supplying the islets of epithelium. The group given heparin 4 hours post-burn showed unequivocal evidence of a better blood supply to the injured tissue, more rapid healing and more extensive healing. Subcutaneous thrombi were diminished.

Since the kidneys of severely burned animals normally show marked congestion, sludging and tubular damage, alternate, lethally burned dogs were treated with heparin. On postmortem examination there was little renal damage in the heparinized animals but necrosis and congestion of the kidneys in the non-heparinized animals.

The two changes in tissue effects of burns, namely, increased healing and less renal damage, point toward the beneficial effects of heparin and warrant further experimental and clinical investigation.

RESPIRATORY SYSTEM

The Influence of Variability in Nasal Mucous Membrane Function on Olfactory Acuity

By *Robert A. Schneider, J. Paul Costiloe, R. Palmer Howard and Stewart Wolf.* Department of Medicine, University of Oklahoma Medical Center, and Oklahoma Medical Research Foundation, Oklahoma City.

Aside from the observation that at the height of a common cold olfactory acuity is poor, no detailed study has been made relating all degrees of nasal function to olfactory threshold level. Variations in olfactory acuity observed in this laboratory in subjects from day to day and between subjects suggested the need for a detailed study of local changes in the nose.

Observations of the color (comparison with Monsell color chart), and degree of secretions, swelling and obstruction (arbitrary scale, 0 to 4+) of the nose and at the same time olfactory acuity (citral) were made 865 times over several months in 5 female and 4 male subjects. Acuity was determined as the smallest amount of odorant introduced into an olfactorium which the subject could detect and identify.

When the values for each of the 4 nasal functions were plotted on a continuum from lowest to highest possible values, each subject could be readily categorized as chronically exhibiting nasal hypofunction (all functions low in value), "average" nasal function or as having nasal hyperfunction (all values high). When the associated acuity levels were considered for each subject at all levels of nasal function, it was found that acuity was best in the midrange for each function, average in the low range of function and poor in the high range. Acuity was therefore best when nasal function was average, and poor when the nasal functions were either low or high in value. Of the 4 functions, variations in color appeared to contribute most to change in acuity, followed, in order, by obstruction, secretions and swelling. We conclude that variations in nasal membrane function do contribute to variability in olfactory acuity.

The Effect of Oxygen on Pulmonary Histology in the Mouse and in Man

By *Ben V. Branscomb, Beulah Hathaway and C. B. McCall.* Departments of Medicine and Pathology, Medical College of Alabama, Birmingham, Alabama.

Evidence has recently been presented in man that oxygen in ordinary therapeutic concentrations can produce in as little as 48 hours capillary proliferation, thickening of alveolar walls and alterations in the small vessels of the lungs. Seventy-six mice were divided into 3 groups. The 1st group breathed room air; the 2nd group, compressed air in a gas-tight tank; and the 3rd, 57 ± 2% oxygen. Oxygen concentration and humidity were monitored continually. Mice from each group were sacrificed at 2-day intervals for 14 days. The remaining mice were then returned to room air and sacrificing continued for 14 days. Sections from the lungs from all animals were examined "blind." No differences in the 3 groups could be detected.

Forty-five consecutive autopsies on adult males were studied as follows: Tissues from the most normal-looking portion of the lung were sectioned and stained. A careful inquiry was made to determine how much oxygen each patient received and by what method. The sections were studied "blind," after which correlations between pathologic changes in the lung and the exposure to oxygen were attempted. There was no increased incidence of alveolar, capillary or vascular change in those patients who received prolonged oxygen therapy with those who received none. The most striking finding was a surprising frequency with which focal areas of capillary proliferation and thickening of the alveolar walls were found in both groups: approximately 85% of the autopsies demonstrated this.

The Effects of Lung Elastin and Surface Forces on the Physical Properties of the Lungs

By *Bill F. Hefley and John A. Pierce.* Department of Medicine, University of Arkansas Medical Center, Little Rock, Arkansas.

The physical properties of the lungs are essential to the maintenance of a normal ventilatory reserve. The factors which influence these properties have been inadequately examined. The purpose of this study was to investigate the influence of lung elastin on the physical properties of the lungs. The effects of surface active forces were also studied.

The pressure volume diagram of the lungs was measured in anesthetized dogs before and after opening the chest. The lungs were excised and extracted in 0.1 N. sodium hydroxide solution for 3 to 5 days. Lung elastin was removed by incubation with crystalline elastase in carbonate buffer solution at pH 8.8, and the pressure volume characteristics were again measured with air inflation.

The mean pulmonary compliance in 8 animals was 32 ± 14 ml./cm. H_2O in the intact animal, 36 ± 13 ml./cm. H_2O with the chest open, and 40 ± 12 ml./cm. H_2O after the lung elastin had been removed. Five of the 8 elastase-treated preparations were studied with saline distention in order to eliminate forces at the air liquid interfaces. Under these circumstances, it was observed that extremely large volumes could be introduced with very small changes in pressure. Compliance approached 1000 ml./cm. H_2O. Examination by chemical analysis revealed that these preparations had a markedly reduced elastin content.

It was striking that removal of the lung elastin failed to alter the volume elastic properties of the lungs with air inflation. Removal of lung elastin markedly altered these properties when the preparations were distended with saline. These findings suggest that the surface active forces in the lungs are of primary importance to the maintenance of normal pressure volume relationships. The fibrous tissue proteins of the lungs, including elastin, serve primarily to provide structural strength to the lungs. These observations are important because they call for a revision of the basic concepts about factors influencing the physical properties of the lungs.

The Mixing of Helium during Rebreathing, and its Relation to the Measurement of Pulmonary-diffusing Capacity

By *Benjamin M. Lewis and Ernest J. Heyford-Welsing.* Departments of Medicine, Wayne State University College of Medicine and Detroit Receiving Hospital.

The rebreathing of a mixture of 0.3% carbon monoxide and 10% helium has proved a useful method of measuring pulmonary-diffusing capacity for CO (Fed. Proc. *11*:95, 1958). When the contents of the rebreathing bag are drawn continuously through an infra-red CO analyzer, the concentration of CO falls exponentially with time after about 10 seconds, both in normal subjects and in patients with emphysema. Analysis of He in the bag at the end of the procedure makes possible the measurement of the volume of the lung-bag system and the calculation of diffusing capacity. By using a mass spectrometer, we have continuously analyzed the He as well as the CO concentration during rebreathing in normal subjects and in emphysema patients. Normal subjects mix the He with the gas in the lungs in 1 or 2 breaths. In emphysema there is continued slow mixing up to 45 seconds of rebreathing. If the poorly ventilated areas in the lungs of emphysema patients had a significant diffusing capacity, CO concentration in the rebreathing bag should not show an apparent exponential fall with time as it does. Consequently, it appears that the poorly ventilated areas of the lung in emphysema have a low diffusing capacity as well.

Lung Volume Changes with Extreme Obesity

By *James K. Alexander, Aubrey E. Guthrie, R. Sakaguchi, H. Wayne Crawford and V. William Cole.* Cardiovascular Laboratory, Jefferson Davis Hospital, and Department of Medicine, Baylor University College of Medicine, Houston, Texas.

To determine the effects of uncomplicated obesity upon lung volumes, and to analyze the mechanisms responsible for these effects, observations were made in some 50 obese patients of both sexes within the weight range of 250–425 lbs. Standard spirographic technics were used and functional residual capacity measured with an open circuit nitrogen washout technic.

Total lung capacity was less than normal, due to reduction in vital capacity, while residual volume remained normal or increased. Reduction in both inspiratory capacity and expiratory reserve volume accounted for the diminished vital capacity. Findings were the same in both sexes, and change from supine to standing position had little effect.

Measurement of thoracic and abdominal girth, together with changes in lung volume, indicated (1) inability of the obese patient to expand the chest during inspiration and (2) that chest expansion per liter of inspired air in the obese state was only a small fraction of the normal. That diaphragmatic action accounted for the change in lung volume to a much greater

extent than thoracic expansion in these patients as compared with normal was further confirmed by fluoroscopic observations. Diaphragmatic movement in the obese was found to be only slightly compromised, while movement of the lower thoracic ribs was reduced to ⅓ the normal. Reduction in total lung capacity was not well correlated with increase in body weight alone, but could be related to the presence of marked lumbar lordosis and thoracic kyphosis associated with obesity, and the comparative sizes of abdomen and thorax. It is concluded that change in lung volumes with extreme obesity is brought about primarily by modifications in the mechanics of thoracic rather than diaphragmatic action, and that these modifications are attributable to postural factors and abdominal size.

Physiologic Alterations Following Tracheostomies for Acute Respiratory Failure in Chronic Pulmonary Insufficiency

By *Josef R. Smith and Watts R. Webb*. Department of Surgery, University of Mississippi Medical Center, Jackson, Mississippi.

The management of acute respiratory decompensation in patients with chronic hypoventilation due to obstructive emphysema, paralytic and other diseases is very unsatisfactory, with a high resultant mortality.

Observations on the physiologic effects of tracheostomy on blood gas and external spirometric values in 10 such patients have given evidence of its value. All patients studied had been resistant to the usual vigorous medical regimen, and 6 who recovered had been considered moribund at the time of tracheostomy. Blood gas studies, pre- and postopertaively, usually showed dramatic improvement with 24 hours. The pCO_2 was usually above 70 mm. Hg preoperatively, and the oxygen saturation markedly diminished even as low as 36%. Preoperative pH values ranged from 6.98 to 7.39, with an average of 7.24. Death occurred from cardiac arrest during tracheostomy in one patient with pH 7.14 and pCO_2 of 105 mm. Hg.

Ventilatory studies showed diminished maximum breathing capacities and increased residual volumes. Comparative studies prior to acute failure and after recovery showed no significant changes. Ventilatory studies were not attempted during acute decompensation.

Follow-up studies up to 18 days postoperatively showed progessive improvement so long as the tracheostomy was open, though none of this severely disabled group was able to reestablish normal values.

Effect of Diamox on Plasma and Urine Acid-Base Composition during Chronic Respiratory Acidosis

By *Norman W. Carter, Donald W. Seldin and Hsi C. Teng*. University of Texas Southwestern Medical School, Dallas, Texas.

Diamox administration to emphysematous patients sometimes lowers arterial pCO_2, perhaps because ventilation improves. To explore Diamox action during respiratory acidosis with relatively fixed pulmonary ventilation, rats exposed to 10% CO_2 were given injections of Diamox every 6 hours for 9 days. The rats were then sacrificed 6 hours after receiving their last dose of Diamox when measurable renal carbonic anhydrase activity was absent. Plasma electrolyte composition, urinary acid excretion and renal enzymatic activity were measured in control animals, animals given 10% CO_2 only and in animals given 10% CO_2 plus Diamox.

Plasma bicarbonate concentration and arterial pCO_2 was 22.3 ± 1.3 mEq./L. and 35 ± 3 mm. Hg, respectively, for control animals; 39.6 ± 2.5 mEq./L. and 102 ± 7 mm. Hg, respectively, for rats given 10% CO_2; 46.6 ± 3.1 mEq./L. and 175 ± 30 mm. Hg, respectively, for rats given 10% CO_2 plus Diamox. Unlike respiratory acidosis alone, Diamox plus 10% CO_2 produced a high plasma chloride (114 ± 2 mEq./L. as opposed to 91.0 ± 3 mEq./L. for 10% CO_2 only). Ammonia was excreted in increased amounts, despite an alkaline urine pH, in the group given 10% CO_2 plus Diamox. Renal glutaminase activity was increased in the group receiving 10% CO_2 plus Diamox, but not in respiratory acidosis alone.

It is concluded that, if ventilation of CO_2 is relatively fixed, Diamox increases arterial pCO_2 by inhibiting red cell carbonic anhydrase. The high arterial pCO_2, in turn, can produce sufficient H^+ via the uncatalyzed reaction in the renal tubular cell to facilitate reabsorption of even increased amounts of filtered bicarbonate. Finally, the elevated plasma chloride could have been the consequence of slight bicarbonate excretion resulting from renal carbonic anhydrase inhibition.

Postoperative Respiratory Acidosis: the Effect of Mechanical Ventilation during Thoracic Surgery

By *Arthur C. Beall, Jr. and R. Maurice Hood*.

Thoracic and Cardiovascular Branch, Surgical Service, U. S. Naval Hospital, Oakland, California.

It is well known that varying degrees of respiratory acidosis occur during thoracic surgery. For this reason, many types of apparatus to inflate the lungs mechanically are now in use in an effort to insure more adequate ventilation throughout operation. It has recently been shown that respiratory acidosis of equal severity may occur during the recovery room period, due to a combination of pain, drug depression and other factors. This investigation was undertaken to determine if a long period of rhythmic, mechanical ventilation during operation tends to depress the respiratory center and enhance the usual hypoventilation seen immediately following operation.

Thirty patients undergoing intrathoracic procedures were studied. Arterial blood was drawn anaerobically the day before surgery, at the end of operation immediately prior to the removal of the endotracheal tube and at 15-minute intervals for the following hour. Determinations of oxygen saturation and carbon dioxide content were made, and the pH was measured. Spirograms were made concomitantly with blood sampling, and tidal volume and minute ventilation were determined.

In no instance was there evidence that a long period of mechanical ventilation during operation was detrimental to the ventilatory adequacy of the patient immediately following operation. There was no statistical difference in arterial blood findings or in ventilation between the manually and the mechanically ventilated group of patients. Individual differences were present, however; the more severe cases of respiratory acidosis, both at the end of operation and in the immediately ensuing period, were found in the manually ventilated group.

These studies would appear to indicate that mechanical ventilation of the patient undergoing thoracic surgery is a safe method of insuring adequate ventilation and does not depress spontaneous respiration following operation.

RHEUMATIC STATES

A Comparison of the Latex Fixation–Whole Serum, Latex Fixation–Euglobulin Fraction and Bentonite Flocculation Tests in the Laboratory Diagnosis of Rheumatoid Arthritis

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There have been recently described several laboratory procedures for the laboratory diagnosis of rheumatoid arthritis. The present study was undertaken to compare the relative sensitivity of the following current tests: (1) latex fixation using whole serum; (2) latex fixation using euglobulin fraction; (3) the Bentonite flocculation test. The subjects investigated were classified as follows: (1) 76 patients with a clinical diagnosis of definite rehumatoid arthritis, (2) 9 patients with a diagnosis of possible rheumatoid arthritis; and (3) 71 patients with musculoskeletal diseases other than rheumatoid arthritis grouped under 11 separate diagnostic categories.

The latex fixation–whole serum test as described by Singer and Plotz gave a 74% of positive reactors in the group of definite rheumatoid arthritis, with none of the atypical rheumatoid arthritics showing a positive reaction. Of the other musculoskeletal disorders only one patient with systemic lupus erythematosus showed a positive reaction. The euglobulin fraction procedure was carried out as described by Craig, Kergy and Parsons. The latex test on this fraction demonstrated an increase in the positive reactors from 74 to 86% in the group of definite rheumatoid arthritis, but again, increases were observed in the other groups of subjects. Employing the Bentonite flocculation test, as outlined by Bozicevich, Bunim, Freund and Ward, an augmentation of positive reactors to 88% was noted for the group of definite rheumatoid arthritis. However, in the remaining group, the following patients also showed positive reactions: 2 with systemic lupus erythematosus, 1 with Sjorgren's syndrome, 2 with shoulder-hand syndrome and 2 with atypical rheumatoid arthritis.

The present studies suggest that these 3 laboratory tests are of value in the diagnosis of rheumatoid arthritis. They are technically easy to perform and give few if any false positive reactions. The Bentonite flocculation test appears to be the most sensitive of the 3 tests evaluated.

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